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Ornamentation, Behavior, and Maternal Effects in the Female Northern Cardinal

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ORNAMENTATION, BEHAVIOR, AND MATERNAL EFFECTS IN THE FEMALE NORTHERN CARDINAL

by

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ABSTRACT

ORNAMENTATION, BEHAVIOR, AND MATERNAL EFFECTS IN THE FEMALE NORTHERN CARDINAL

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This study seeks to understand the relationship between ornamentation, maternal effects, and behavior in the female Northern Cardinal (*Cardinalis cardinalis*). Female birds possess ornaments that indicate a number of important known aspects of quality and are usually costly to maintain. However, the extent to which female specific traits, such as maternal effects, are indicated is less clear. It is predicted by the Good Parent Hypothesis that this information should be displayed through intraspecific signal communication. Specifically, androgens and carotenoids are of interest in this study because both are linked to ornamentation, and are also important egg components that impact offspring quality. Additionally, androgens have implications for adult behavior; testosterone specifically is well known to affect aggression. However, results from this study do not support these hypotheses. Instead, no association was found between ornamentation and maternal effects or behavior. We suggest that it is not profitable for signals to be maintained if the cost of maintenance of such traits is more than the value of the information they display. This research indicates that sexually selected traits should be looked at in the context of the organism's life history in order to determine functionality.

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CHAPTER I

INTRODUCTION: ORNAMENTATION AND THE NORTHERN CARDINAL

Ornamental plumage, conspicuous colors, and other visual signals in animals have historically been the subject of much study and debate (Andersson 1994). Animals across all taxa commonly display traits that cannot be explained by natural selection. The existence of characteristics that seemingly convey no survival advantage was a quandary for early evolutionary biologists. Wallace (1872), Poulton (1890) and Darwin (1871) all had early competing theories on the relevance and function of these traits. Wallace claimed that conspicuous traits function primarily as warning signals, and did not accept Darwin's theories that mate choice and competition could act as selective pressure. Poulton erroneously supported Wallace's opinions on warning coloration and also claimed that bright coloration could evolve as a signal to benefit predators. Both authors addressed part of the problem of conspicuousness in traits by acknowledging that some brightly colored individuals display warning information, but they did not address the problem in full. Primarily, why there is a difference in many conspicuous traits in male and female individuals, why many traits are used in competitions for mates, why only sexually mature organisms usually display full expression of the trait, and why non-toxic organisms display conspicuous or colorful traits. Although much debate remains surrounding mechanisms and functions, Darwin's theory prevailed and simply states that sexually selected traits are a result of differences in reproductive success caused by competition over mates. Darwin revealed a pattern in certain conspicuous traits that he considered the result of what he termed sexual selection (see Andersson 1994):

1. Traits should not be acquired before sexual maturity.

2. Females should develop the trait to a lesser degree than males.
3. Males should only develop or display the trait when mating can occur.
4. The trait is displayed to potential mates or sexual rivals (i.e., members of the same species).

Indeed, many of the conspicuous traits seen in animals fit this pattern. The colorful plumage of birds grown during the breeding season, and the antlers grown by male deer during the breeding season are both good examples of such traits.

Points one and two describe *who* will develop these traits, primarily sexually mature males, and to a lesser degree sexually mature females. Only sexually mature individuals compete for reproductive opportunities because a conspicuous ornament conveys no survival advantage, and no reproductive advantage if the individual is incapable of reproducing. It is generally true that females develop these traits to a lesser degree; however, there is a great depth in the variety of sexual dimorphism seen in different organisms. There is considered to be less competition for mates among females, largely because of their greater inherent reproductive success (Andersson 1994). The relative scarcity of female gametes when compared to male gametes leads to the generalizations of choosey females and promiscuous males (Andersson 1994). As such, the competition to breed is greater among males, leading to heightened expression of these traits. Points three and four explain *why* individuals have these traits: in order to present information relevant in the competition for reproduction in the correct context to mates or rivals. A more proximate explanation is that differences in certain physical traits and behavior that differ between males and females are the result of different levels of sex hormones associated with the two types of gonads and gametes. Thus, hormones

can serve as a potent influence on the development of these secondary sexual characters, as well as behavior associated with their display.

Hormones

Evolution has produced physiological differences in males and females, which result in the dimorphism seen in secondary sexual traits. Hormones have been investigated rigorously as the mechanism through which secondary sexual characteristics become enhanced for many years (Adkins 1975; Hutchinson 1978; Adkins-Regan 1987). Testosterone (T) is often seen as the "male" hormone, while estrogen (E) is usually thought of as the "female" hormone. This is because each hormone is not only required for proper production of respective gametes for each sex, but also the secondary sex characteristics resulting from the struggle to reproduce, as mentioned above. Without exposure to correct sex hormones during development, proper sexual differentiation does not occur later in life, nor does normal production of gametes occur (Adkins-Regan 1987); T-implanted females do not develop properly, nor do estrogen-implanted males. Hormonal influence during development also helps create "male" and "female" brains (Balthazart and Ball 1995), (which has implications for behavior), as well as organizing pathways and receptors that will be used once sexual maturity is achieved (Adkins-Reagan 2005). Hormonal control of these processes is strong, as removal of gonads inhibits sexual differentiation, and when supplemented with the opposing sexes' hormones, genetic males can develop female sexual traits, and females can develop male-like traits (Adkins 1977; Adkins-Regan 1987; Hutchinson 1978; Balthazart and Ball 1995). At the onset of puberty, hormonal axes come "online" and facilitate the production of an appropriate ratio of male or female hormones from the gonads, which

can then impact adult sex-specific behavior and appearance. Therefore, genetic differences in male and female animals manifest as hormonally driven divergences between male and female morphology at puberty, and the study of sexually selected traits is fundamentally a study of the proximate differences in mechanisms between male and female. However, within each sex there is a wide variety of "maleness" and "femaleness" which can be expressed. At the proximate level, it is understood that varying levels and ratios of T and E often account for these differences. However, why sexual selection produces such variety at the ultimate level has been addressed by a number of hypotheses.

Ornament Hypotheses

The traits resulting from Darwin's struggle for reproductive success are usually termed "ornament" or "armament." The term "ornament" generally refers to a trait not specifically used in the survival of the individual, but rather in mate selection, as opposed to direct competition or fighting, while "armaments" are usually used as weaponry in competition for mates (Andersson 1994; Amundsen 2000). Since Darwin (1895) published his theory on sexual selection, a multitude of hypotheses have been put forward to explain the complexity and magnitude of the variation we see in the evolution of secondary sex characteristics and mating tactics in animals.

Darwin recognized avian ornamentation in particular because bird species commonly have very exaggerated ornaments and are often high sexually dimorphic. As such, many of the hypotheses about how these traits are formed and function have used birds as a model group. Darwin hypothesized that certain male traits may become exaggerated by female preference. Darwin's theories on sexual selection stemmed

further research by Fischer (1930) into the area of female preference. Fischer showed that females could, theoretically, develop a genetically linked preference for male ornamentation that would self-reinforce the trait. The choice behavior leading to elaboration of traits is what Fischer termed a "run-away" process (e.g., the trait can be quickly pushed to maladaptive expression in terms of survival and longevity). The conspicuous ornaments seen in some birds are a result of this "run-away" process; Common Peafowl ("peacock" *Pavo cristatus*) with their greatly elaborated train (e.g., the fan of tail feathers displayed) are a well-known example of this. In organisms in which this process occurs, an ornament can quickly become meaningless, as it's expression becomes genetically "fixed" in the population.

Various hypotheses have been developed to attempt to explain what type of information is conveyed in ornaments, whether signals are honest indicators of quality and how "cheaters" are eliminated. The Handicap Hypothesis proposes that females prefer conspicuous sex characteristics that act as a handicap to the bearer (Zahavi 1975). Only high quality individuals should be able to survive natural selection with exaggerated traits used only in mate attraction (Zahavi 1975). The Good Genes Hypothesis proposes that superior genes are the mechanism responsible for this ability of individuals with conspicuous sex characteristics to survive the impacts of natural selection (Hamilton and Zuk 1982). However, the variation seen in ornamentation within a species could not be explained by inheritance, and ornaments have also been theorized to be condition dependent indicators of quality (Andersson 1986), meaning that greater expression of an ornament indicates better health and condition, implying a more high quality individual. Benefits for mating with such an individual may be the result of direct (genetic) or

indirect (resources) benefits. The Good Parent Hypothesis suggests that sexually selected traits may indicate indirect benefits to the offspring that an individual can provide, such as feeding rates (Hoelzer 1989), and this could perhaps be extended to an earlier stage called maternal effects for females (Jawor et al. 2004).

Additionally although much research focuses on a single ornament, many species have multiple ornaments. More than one selective pressure may help shape ornament expression, and many species have more than one ornament that may be used to convey information or be a focus of mate choice (e.g., having ornaments which show genetic quality and condition-dependent quality). Møller and Pomiankowski (1993) presented three hypotheses about the use of multiple ornaments in sexual selection.

- The Multiple Message Hypothesis states that all ornaments convey discreet information about the condition or behavior of the individual.
- The Redundant Signal Hypothesis states that ornaments convey redundant information by conveying the same information multiple times.
- The Unreliable Signal Hypothesis states that only one ornament is still a reliable signal conveying true information, other ornaments no longer reliably indicate information on quality.

However, models predict that most ornaments should be unreliable, and in multiple ornament systems only one ornament should be most reliable in how it conveys information on its bearer (Schluter and Price 1993). Although the Multiple Receiver Hypothesis suggests that when multiple messages display different or similar information to mates and competitors, all ornaments can be reliable indicators of quality (Andersson et al. 2002). For example, Red-collared Widowbirds (*Euplectes ardens)* have a red

feather patch on the neck that is subject to intrasexual selection, and greatly elongated tail feathers which are subject almost exclusively to intersexual selection (e.g., information given to multiple receivers). Additionally, when different physiological processes are involved in different ornament production pathways; this may allow multiple ornaments to indicate very different aspects of physiology. For example, androgen levels mediate expression of melanin ornaments, and both androgens and melanin ornaments are known to indicate social status/dominance (McGraw 2006). Carotenoid ornaments are considered to be condition dependent, and therefore may convey more variable aspects of quality, such as health and access to resources, similarly, this information may be perceived differently by mates and competitors (McGraw and Hill 2000).

Melanin Ornaments

Melanin pigments are produced by melanosomes in the skin from diet-obtained, non-essential amino acids, L-tyrosine or cystein (Jawor and Breitwisch 2003). However, there is conflicting evidence on whether or not melanin ornaments are strongly influenced by diet (Hill 2000; but see Jawor and Breitwisch 2003). The amino acids necessary to create melanin pigmentation may be food derived, but the ability to convert them into melanins is thought to have a genetic component (reviewed in Jawor and Breitwisch 2003). Therefore, expression of melanin ornaments is thought to be less condition dependent, and more dependent on good genes (Jawor and Breitwisch 2003). The expression of melanin ornaments has been shown to be associated with androgens, and androgen receptors are known to exist on melanocytes (Jawor and Breitwisch 2003). There is a well-studied link between aggression, melanin ornamentation, and T; as T has been shown to make birds both darker in color and more aggressive. Originally, melanin

ornaments were thought to be "cheap" to build and maintain, however more recent studies do no support this (reviewed in Jawor and Breitwisch 2003). The social aspect of these ornaments may help to keep them honest, as birds with dark melanin ornaments must assert themselves to maintain dominance (Senar 2006). Additionally, melanogenesis itself may be costly because of the large number of free oxygen radicals created during this process that must be neutralized or deactivated, which is typically done via diet-based carotenoids (von Schantz et al. 1999). Consequently, the radicals created may negatively affect the availability of carotenoids (von Schantz et al. 1999). In contrast to carotenoid ornaments melanin ornament expression seems to be largely independent of parasite load (McGraw and Hill 2000). Finally, T may act on the development of melanin ornaments in offspring, giving them a mating advantage later in life (reviewed in Jawor and Breitwisch 2003). Taken together, evidence indicates that melanin ornaments indicate genetic quality, behavior, and to a lesser degree condition (at least indirectly).

Carotenoid Ornaments

Carotenoid pigments cannot be synthesized *de novo* by animals, and therefore must be obtained from the diet; although, simple carotenoids like β-carotene can be modified into more complex xanthophylls in the liver (reviewed in McGraw 2006). Hence, carotenoid ornaments may be an indicator of liver function. Carotenoids are considered to be condition dependent indicators of quality (Hill 1990), and a wide degree of variation is often seen within these ornaments. Birds must find and modify carotenoids, which also have a wide variety of uses in the body beyond plumage coloration. Parasite load is known to inhibit carotenoid ornament expression, via

interruption of carotenoid absorption from the intestines, and has therefore been suggested to be an indicator of parasite resistance (Hamilton and Zuk 1982). Additionally carotenoids quench free oxygen radicals (von Schantz et al. 1999). These radicals are a by-product of cellular metabolism and immune function. Unhealthy animals produce large quantities of radicals, which require a large amount of carotenoids to deactivate. Female birds have the additional task of loading eggs with carotenoids to quench the numerous free-oxygen radicals that result from mitotic division during embryological development. The wide variety of uses in the body for carotenoids may help to keep this condition-dependent ornament honest. Birds with less expression of carotenoid ornaments are found to be in poorer health, with greater occurrence of disease and parasites (McGraw 2006). Birds must preferentially use carotenoids to maintain health; only birds with an overabundance of carotenoids will be able to build them into feathers or eggs.

Female Ornaments

Until recently female ornamentation was considered an unavoidable by-product of a shared genome, and as a result were thought to have no functional use (Lande 1980). Female ornaments have since been linked to aspects of quality and behavior just like male ornamentation (Amundsen 2000; Amundsen and Pärn 2006). Whether female ornamentation displays inter- or intraspecfic information varies between species (Amundsen 2000). It may be advantageous for males to determine the quality of females from ornamentation, particularly in mating systems in which males contribute significantly to offspring care (Amundsen 2000; Chenoweth 2006).

Although ornaments in females have historically received less attention, recent research has begun to show that these ornaments indicate aspects of condition and quality in females rather than just being genetically linked (reviewed in Amundsen 2000; Amundsen and Pärn 2006; Lande 1980). While these studies often show correlations between female ornamentation and certain aspects of condition or quality, the traits (both ornament and condition) assessed are usually not unique to females, even though female birds undergo very distinct physiological changes, and can play a large role in the development of offspring through maternal effects and behavior. For example, in the Northern Cardinal (*Cardinalis cardinalis*), females participate in territory defense alongside mates, must lay eggs and incubate clutches, and defend clutches from predators and other females (Halkin and Linville 1999). The consequences of inadequacies in these areas are severe, resulting in potentially no reproductive success for either pair member. It is possible that female ornaments in cardinals, which have been found to indicate intrasexual aggression (Jawor et al. 2004) may also indicate information to males about the quality of maternal effects (egg components) and maternal behaviors (Jawor et al. 2004).

The Northern Cardinal

The Northern Cardinal is a prolific, non-migratory songbird of the eastern United States, Mexico, southern Canada, and parts of Central America (Halkin and Linville 1999). Its range is currently expanding northward as it takes advantage the fragmented habitat created by urban expansion, a lifestyle to which is it well suited as an edge-nesting grainivore (Halkin and Linville 1999). Its abundance makes the cardinal convenient to study, while its ecology and physiology make it an interesting and unique

research model. Cardinals are socially monogamous, bi-parental and possess multiple ornaments, including unique female ornamentation (Jawor and Breitwisch 2004; Jawor et al. 2004). During the pre-breeding and breeding seasons both males and females sing, actively defend a territory and together raise multiple broods of offspring from spring to late summer (Halkin and Linville 1999). Females alone incubate eggs, while males do the majority of nestling feeding and care of fledged offspring. Because participation in parental care by both members of the pair is required for successful breeding, it follows that each member would benefit from being with a mate of high genetic quality, and/or an individual capable of contributing indirect benefits (resources).

There are two derivations of plumage color in cardinals, which are based on two different pigment classes: carotenoids and melanins. The dark facemask females and males possess is melanin-based. The female facemask is smaller, and generally lighter (although this ornament is highly variable in females [see Figure 1]). Females also possess carotenoid-based red under-wing coverts (see Figure 2), which are behaviorally displayed to males and females (Jawor et al. 2003; Jawor et al. 2004), and a carotenoid pigmented bill. It is thought that the two ornament types can display unique information because of the vastly different ways in which they are obtained and used in the body. In cardinals, female ornamentation has been shown to be indicative of reproductive success, aspects of condition, timing of first nest, and aggression levels (Linville et al. 1998; Wolfenbarger 1999; Jawor and Breitwisch 2004; Jawor et al. 2003, 2004). Repeated aspects of condition were indicated by multiple carotenoid ornaments, which supports the Redundant Signal Hypothesis (Jawor et al. 2004); however, different information was displayed by carotenoid and melanin ornaments (aggression, nest provisioning etc.),

supporting the Multiple Messages Hypothesis. Because female cardinals have multiple ornaments that co-vary with different aspects of quality, different information may be interpreted differently depending on the receiver. This may allow a single or multiple ornaments to provide different information to potential mates verses potential rivals (McGraw 2006). It is possible that female facemasks/underwings in cardinals display information that would be both attractive to males, and intimidating to female rivals. Female cardinals play a significant role in territory defense in the pre-breeding and breeding season (DeVries et al. unpubl. data). In circumstances in which females gain an advantage by pairing with high quality mates, it is likely that females compete over males and resources (Langmore et al. 1996). Given the multiple functions of the components that help build these color ornaments, it is conceivable that the same or different information given by one or more ornaments would be beneficial to both mates and rivals. It is also possible that females display information consistent with the Good Parent Hypothesis by advertising the ability to stock eggs with important components for embryo development.

With the Northern Cardinal as a model species, I am most interested in testing the Good Parent Hypothesis (Hoelzer 1989), but moving the predictions of the hypothesis to an earlier state of parental care, the provisioning of eggs by females with steroid hormones and carotenoids needed for embryonic development called maternal effects. Female Northern Cardinals have informative carotenoid and melanin ornaments, both of which are known to be affected by two very important components that are also used in eggs (T and carotenoids). If a female retains high levels of these compounds in her body,

it is predicted that they will be deposited into both eggs and into ornaments to display this ability to mates.

Research has shown that the ornaments of female cardinals may display the same information regarding condition in different ornaments (Redundant Signal Hypothesis), as well as different information, such as behavior (Multiple Messages Hypothesis) (Møller and Pomiankowski 1993; Jawor et al. 2004). Female cardinals have been shown to indicate intrasexual aggression with melanin ornaments in a Northern population. I am interested in replicating this study in a Southern population. If the same ornament indicates different information to males (Good Parent Hypothesis) and females (intrasexual aggression), implications from this study could suggest support for the Multiple Receivers Hypothesis (Andersson et al. 2002), as females may display different types of information to males (i.e., ability to provision eggs), and females (i.e., intrasexual aggression level).

Figure 1. Facemasks in Female Northern Cardinals, Showing Variation in Expression and the Scoring System.

Figure 2. Female Northern Cardinal Carotenoid-Based Underwing Ornament.

CHAPTER II

ORNAMENTATION AND MATERNAL EFFECTS IN THE FEMALE NORTHERN CARDINAL

Introduction

Birds are often used as models to investigate the relationship between sexually selected traits and aspects of quality because of the obvious ornaments males of many species display (Andersson 1994; Amundsen 2000). Common and strong sexual dimorphism has lead to mostly male dominated research leaving female ornamentation largely ignored. Yet, female ornaments have recently become increasingly recognized for their ability to indicate aspects of quality similar to males (Amundsen 2000; Roulin et al. 2001, 2003; Jawor et al. 2004; Amundsen and Pärn 2006; Hanssen et al. 2006). More highly ornamented females have been shown to be more aggressive (Hegyi et al. 2008; Murphy 2009a, b), and to be preferred as mates (Jones and Hunter 1993, 1999; Amundsen and Forsgren 1997; Griggio et al. 2005). Most aspects of quality investigated in these studies are not female specific, and yet females have a very unique role during reproduction in many taxa. In egg-laying animals, yolks must provide developing offspring with all the essential nutrients for rapid development; carotenoids, vitamins, proteins, fatty acids, and androgens such as T are all important components (Blount et al. 2000). Many studies investigating this topic with bird models test more general aspects of maternal investment, such as laying date, clutch size and success (Parker 2002; LeBas et al. 2003; Weiss et al. 2006; Doutrelant et. al 2008; Hargitai et al. 2005). A study with the Northern Cardinal has shown that ornaments of females of this species indicate quality similar to that of males, as well as aspects of maternal investment, such as laying

date, reproductive success, and feeding rates (Jawor et al. 2004). However, studies with other species show no relationship between maternal investment and ornamentation (Rohde et al. 1999; Komdeur et al. 2005; Murphy 2007), leaving the prevalence of maternal quality displayed in ornamentation uncertain. Studies have shown ornaments indicate maternal effects in a variety of non-avian taxa from carotenoid load of eggs in fish (Massironi 2005; Svensson et al. 2006), to yolk size in lizards (Weiss et al. 2006); however, studies specifying which ornaments in birds indicate more direct (not to be confused with direct genetic benefits [Weatherhead and Robertson 1979]) maternal effects, such as egg components, are rare (McGraw et al. 2005). The lack of studies is surprising, given a female's ability to enhance the fitness of both members of the pair through egg provisioning (Blount et al. 2002). Males cannot contribute directly to offspring development until after hatching, and it should be beneficial for them to determine a female's capacity to produce high quality eggs and offspring, particularly in monogamous, bi-parental birds in which the male invests heavily in offspring with one female (Blount et al. 2000; Amundsen and Pärn 2006). Males may obtain fitness benefits by greater offspring survival through their choice of females, if there is variation in female reproductive success or genetic quality (Parker 1983; Owens and Thompson 1994; Chenoweth 2006). Additionally, comparing maternal effects to female ornamentation may be thought of as an extension of Hoelzer's Good Parent Hypothesis, in which individuals choose mates that will increase their own fitness through their mate's ability to provide parental care (Hoelzer 1989; Jawor et al. 2004). Here I investigate maternal effects in eggs, as these components come directly from the female,

and indicate the female's ability to provide a unique type of parental care and whether they co-vary with ornamental expression.

Maternal Effects

Maternal effects are all non-genetic materials introduced to developing offspring through the female by way of the egg yolk. These materials commonly include fats, proteins, vitamins, and hormones (Blount et al. 2002). Maternal effects are beneficial to offspring, and they are a way for the mother to implement developmental changes in order to adapt and shape the phenotype of the offspring to fit the environment into which it will hatch (Mousseau and Fox 1998). These changes can have long-term effects on morphology, behavior and life-history strategies (Gil 2003). Egg components are known to influence offspring long after development as well, having far reaching impacts on health and phenotype of adult offspring, thereby enhancing fitness of the parents (Saino et al. 2007; Biard et al. 2009).

Good Parent Hypothesis

The Good Parent Hypothesis states that individuals who choose mates based on information displayed about parental care can achieve greater reproductive success, through improved phenotype of the offspring (Hoelzer 1989). Although the Good Parent Hypothesis is more commonly attributed to male parental effort, female non-heritable qualities should be of equal importance in monogamous mating systems where parental care from both parents is required for offspring survival (reviewed in Amundsen 2000). It is important for the reproductive success of both members to the pair that the female produce high quality eggs provisioned with many nutrients. Because of the closed nature of the egg, this nutrient transfer occurs over a small window during the female's

reproductive cycle; a low quality female (i.e., poor health or genes) should not be able to provision eggs as well as a high quality female during this timeframe (Gil 2003). T and carotenoids are the components of most interest in this study. While these components do not encompass all the important maternal effects that enhance offspring development, they are considered here because both have a strong association with ornament expression in the study species, as well as being vitally important for proper development of the embryo (Gil 2003).

Testosterone

T is a well known, and much studied egg component (Groothuis et al. 2005). It is known to have anabolic effects, and T in eggs is well known to have a positive effect on development at optimal levels (Blount et al. 2000; Groothuis et al. 2005). T is known to enhance development and some research shows that T actually increases immune function in offspring (Andersson et al. 2004; Navara et al. 2006), and can have far reaching implications for health and behavior (Møller et al. 2005). Additionally, T is known to increases bioavailability of carotenoids (Blas et al. 2006), which enhances immune function (Blount et al. 2000), making the association between these two maternal effects components complex. T has been shown to help development of the hatching muscle in chicks (Lipar and Ketterson 2000), and hatchling growth (Schwabl 1996), giving chicks from eggs with elevated T a survival advantage. T is also known to affect melanin ornament expression (Jawor and Breitwisch 2003; Bókony et al. 2008). Individuals with darker melanin badges have higher levels of T (Rohwer 1978), and supplemental T makes melanin darker. The dietary component is not a strong influence with melanin ornament expression, as it is with carotenoid ornament expression (Jawor

and Breitwisch 2003). Control of T provisioning in the egg is not well understood, and is generally considered to be under passive control (Schwabl 1996). Therefore, females with high T levels, or frequently elevated T levels during vitellogenesis conceivably put more T into their eggs.

Carotenoids

Carotenoids are a diet-based compound that can be modified in the liver, but cannot be made *de novo* (Goodwin 1984). Because of this, they represent a resource that is considered to be limited, and of varying availability among individuals. They are of vital importance for the health of an adult bird, as well as a healthy egg yolk. Additionally they are used in feather growth during molt in sexually selected ornaments in many species (reviewed in McGraw 2006). Carotenoids in eggs are known to enhance immune function in both adults and offspring (Blount et al. 2000). However, perhaps the most important role of carotenoids in the egg are as free oxygen radical scavengers; this helps reduce oxidative stress that can accrue from rapid development (Blount et al. 2000). Free oxygen radicals are by-products of metabolism, they are single oxygen molecules that are unstable, and therefore seek to join with other molecules, thereby altering those molecules; this can have a profound effect on lipid bilayers, DNA, and other sensitive tissues in the body (Chew 1996). Because the egg is a closed system, free oxygen radicals from fast development have nowhere to go, and therefore mitigation of their damage is achieved by stocking egg yolks with many carotenoids (Blount et al. 2000). This ensures proper development, and minimal damage to sensitive embryonic tissues (Blount et al. 2000). The amount of carotenoids a female puts into eggs is related to environmental availability, as well as her current parasite load and health (McGraw

2006). Because carotenoids are a limited resource, and utilized by both mother and embryo, levels in the egg reflect a maternal trade-off. Carotenoids used to put into egg yolks cannot be utilized by the mother to mitigate her own oxidative stress due to illness, parasites, or other functions that may require excessive mitotic division (Blount et al. 2000). Therefore, whether a female has the resources to provision eggs with high levels of carotenoids can indicate her quality and current health. More "high quality" individuals are expected to have more carotenoids available to put in eggs because they have better territories, with better access to those resources, or because they are healthier. Additionally, carotenoids and T may represent a balancing act, whereby super or sub-optimal levels of T are mitigated by carotenoid deposition (Royle et al. 2001).

Egg Order

Birds often put varying amounts of both carotenoids and T in eggs (Blount et al. 2000; Gil 2003). This variation can occur both between individuals, and between clutches of a single individual (Schwabl 1993). This is thought to occur to correct differences in development time due to asynchronous laying (Gil 2003). Most bird species in which there is a significant laying order show a pattern of increasing T through laying (Schwabl 1993; Lipar et al. 1999; Eising et al. 2001), however, many birds show no significant laying order (Ellis et al. 2001; Wittingham and Schwabl 2002). Carotenoid patterns often mimic those of T (Török et al. 2006), and carotenoids are thought to function to mitigate additional oxidative stress accrued from increased T levels in the egg.

Study Species

The Northern Cardinal was chosen as a model species for this study. Ornaments in female cardinals have been shown to indicate laying date, nestling feeding rates, aggression levels, and body size (Jawor et al. 2004). Cardinals are an excellent candidate species for this work, as females are already known to have informative ornaments that communicate maternal investment potential (Jawor et al. 2004). It has been suggested that female ornaments may indicate maternal effects as an extension of the Good Parent Hypothesis (Jawor et al. 2004; Hoelzer 1989). Male cardinals should have an interest in a female's ability to produce high quality eggs because as a bi-parental species, her investment will affect his fitness (Blount et al. 2000; Amundsen and Pärn 2006). Males may ascertain this information through informative ornaments. Female cardinals have both melanin-based ornaments (facemask see Figure 1) and carotenoid based ornaments (underwing see Figure 2), both of which are highly variable (Jawor et al. 2004). Prior research has shown a high level of carotenoids and T (which is known to affect ornamentation) in egg yolks (J.M Jawor, unpubl. data).

Here I wish to indirectly test whether female cardinal ornaments can indicate maternal effects as an extension of the Good Parent Hypothesis by determining whether T and carotenoids in eggs co-vary with two female ornaments; the melanin-based facemask and the carotenoid-based underwing coverts. Additionally, I wish to determine whether a laying order is observed in this species, as this may have implications for mechanism and hormonal control relevant to other studies.

Materials and Methods

Field Methods

This study was conducted over a two-year period from 2009-2010 at the Lake Thoreau Environmental Education and Research Center property owned by the University of Southern Mississippi (USM), a 131-acre site in Hattiesburg, Mississippi.

Females to be included in the study were passively caught through the non-breeding and early breeding season with mist-nets and potter traps, and banded with a USFWS numbered stainless steel band, and three unique color bands for identification. During the breeding season territories were identified by finding nests, which were usually occupied by a banded member of the population. If a nest was found that was not occupied by a known individual, the female was caught for ornamental measures after egg laying, but before incubation of the nest began.

Ornaments

Either during passive capture or target netting, carotenoid and melanin ornaments of females were measured with an Ocean Optics USB4000 color reflectance spectrometer with a D-2000 Deuterium UV-VIS light source. The probe was placed lightly against the feathers of the ornament to be measured to determine reflectance. Carotenoid ornaments were measured twice and spectral data from these measures averaged. Melanin ornaments were measured from the same location under the bill on each bird.

Egg Collection

Territories of banded females were determined prior to and into the breeding season. Pairs begin breeding in this population in early April. Nests usually consist of 2-3 eggs and pairs nest repeatedly throughout the breeding season. When a nest was found,

eggs were marked each day after laying (for laying order), and were left in the nest until all eggs were laid. A total of 19 nests were included in the study. First clutchs for each female were collected to avoid temporal variability due to differences in nutrient (specifically carotenoids) availability throughout the season. Eggs were frozen (-20°C) until analysis.

Ornament Analysis

Spectral data were analyzed following the completion of fieldwork. Because color is based on three separate dimensions, hue, saturation, and brightness all three must be determined separately (this is only of significance with carotenoid ornaments). Hue was estimated as the wavelength at which reflectance is halfway between its minimum and maximum λ[R50]. Saturation was estimated as the difference between two spectral segments, with the segment divider defined as λR(50). The difference was divided by total reflectance. Brightness was estimated as the total reflectance between 400 and 700 nm. The hue, saturation, and brightness scores were entered into a principal component analysis to compress the score for carotenoid ornaments. For melanin ornaments, only brightness was determined (J. Endler pers. comm.) by averaging reflectance from 400 to 700nm.

Lab Methods

All lab work was performed at the University of Southern Mississippi. Androgens (specifically T) from egg yolks were quantified in Dr. Jodie Jawor's lab, while yolk carotenoids were quantified in Dr. Kevin Keuhn's Lab. Egg yolk samples were prepared by removing albumin and shell layers from frozen eggs until only yolk

remained. Yolks were weighed in grams to the nearest 2 decimal places, and each was placed in a 1.5 ml eppendorf tube and homogenized.

Egg Androgen Analysis

Steroids were extracted from egg yolks samples by dissolving a small amount (12 mg) of yolk in 500 µl of dH_2O. Radio labeled T (2000 counts per minute [cpm]) was added to determine recovery efficiencies. Additionally, standards and blanks were made as positive and negative controls within the assay. For extraction of steroids, 3 mL of a 30:70 mixture of petroleum ether and diethyl ether was added to the yolk solution and the phases were allowed to separate for 20 minutes, this was repeated 3 times, and ether was allowed to evaporate. Samples were reconstituted in 1 mL of a 90:10 ethyl alcohol dH_2O mixture, and were then centrifuged at 2000 rpm's for 5 minutes to remove flocculent proteins. The ethyl alcohol:dH_2O mixture was allowed to evaporate, and samples were then reconstituted in 50 µl of 100% ethyl alcohol and 300µl of buffer, and run on an ELISA Immunoassay (EIA) testosterone plate from Assay Designs (Ann Arbor, MI). In this assay a series of antibodies are used to capture T in the samples to the plate, and levels are quantified using sandwich antibodies and a color change tag. The percent recovery from extractions was determined through a known amount of radioactive T added to the samples and recovered at the end of the assay. Preliminary work has shown that this method is successful at quantifying egg yolk steroids in cardinals (J.M. Jawor, unpubl. data).

Egg Carotenoid Analysis

Carotenoids were analyzed following the methods in Surai and Speake (1998). Briefly, an aliquot of yolk (0.2-0.5g) was homogenized in 2 mL of a 1:1 (v:v) mixture of

5% NaCL solution and ethanol. Carotenoids were extracted from egg yolks using hexane, and reconstituted in a 50:50 mixture of methanol and chloroform, this ratio ensured both carotenoids were thoroughly dissolved. After centrifugation for 5 minutes at 12,000 rpm's, hexane extracts were combined and evaporated under N_2, the residue was dissolved in 1 mL of methanol: dichloromethane (1:1, v:v) and centrifuged. The supernatant was used for carotenoid determination.

Canthaxanthan and β-carotene were quantified by High Pressure Liquid Chromatography (HPLC) using a Kinetex 2.6μm C-18 column (4.6 x 150 mm, Phenomenex Inc.), maintained in a Shimadzu column oven (CTO-10AD) and a Shimadzu liquid chromotograph system (Pumps LC-10AT, Controller SCL-10A). Isocratic runs were performed using a mobile phase containing 70% methanol and 30% chloroform at a flow rate of 1.0 ml min-1. Canthaxanthan and β-carotene were detected at 470 nm using a Shimadzu (SPD-10A) UV/VIS detector (retention time, canthaxanthan = 1.95 min and β-carotene = 3.20 min), and were identified and quantified based on comparison with known canthaxanthan and β-carotene standards (Sigma Co.). Preliminary work has shown that this method is successful at qualifying and quantifying egg yolk carotenoids in cardinals (J.M. Jawor, unpubl. data).

Statistical Analysis

Statistical analyses were conducted in JMP 8 (2009 SAS Institute Inc.) for Spearman rank correlations, ANCOVA's and descriptive statistics (Table 1). For principal component analysis SPSS 9 (2009 SAS Institute Inc.) was used. Data were not normally distributed, but the tests used were considered robust enough to accommodate the data; when deemed appropriate, non-parametric tests were used (Spearman rank

correlation). Because hue, saturation and brightness each explain a part of the color of an ornament, principal component analysis was used compress these three scores; hue and brightness were retained together, and saturation was not. Therefore, there are two scores to describe plumage coloration, a hue/brightness score and a saturation score.

Results

Egg Yolks

Because clutches were collected over two years (2009 and 2010), and clutches contained anywhere from 1-3 eggs, we first tested for effects of year, egg number and individual female on carotenoid and testosterone concentrations in the egg. No year effect was found with the carotenoid concentration in eggs (Table 2). There was a year effect of T concentration in the eggs, however, there was also a strong individual effect, and individuals were never used twice in both years, therefore this may be an artifact of individual differences (Table 3). Although T in eggs varied significantly depending on the individual, carotenoid concentrations did not (Tables 2 and 3).

There was no egg order effect for T concentration (Table 3 and Figure 4), however, there was a near-significant suggestion that egg carotenoid means are not the same in all three eggs (Table 2 and Figure 3). A post-hoc test revealed that first eggs had slightly more carotenoids than later eggs (See Tables 1, 2 and 3). Additionally, although two carotenoids were quantified by HPLC, only β-carotene was present at detectable level.

Table 1

Showing Descriptive Statistics for Ornaments and Egg Components

	N	Mean	SD
Hue/Brightness	19	5.262	±0.999
Saturation	19	5.263	±1.000
Mask Melanin	19	6.7826	±6.9889
Egg (1) T	21	54.854	±32.329
Egg (2) T	20	62.408	±36.607
Egg (3) T	15	68.411	±30.252
Egg (Avg.) T	21	61.987	±31.677
Egg (1) Car.	21	16.133	±4.932
Egg (2) Car.	19	13.783	±4.419
Egg (3 Car.)	14	12.559	±4.627
Egg (Avg.) Car.	21	14.509	±3.606

Table 2

ANCOVA Model Comparing Dependent Variable Egg Carotenoid Levels to Fixed Factors Egg Number, Bird ID, and Year

	df	F	P
Egg Number	2, 29	3.2199	0.0546
ID	17, 29	1.8522	0.0699
Year	1, 29	0.0205	0.8872

*Denotes significant results.

Table 3

ANCOVA Model Comparing Dependent Variable Egg Testosterone Levels to Fixed Factors Egg Number, Bird ID, and Year

	df	F	P
Egg Number	2, 29	0.3714	0.6930
ID	*17, 29*	*4.03004*	*0.0003**
Year	*1, 29*	*4.8420*	*0.0359**

* Denotes significant results.

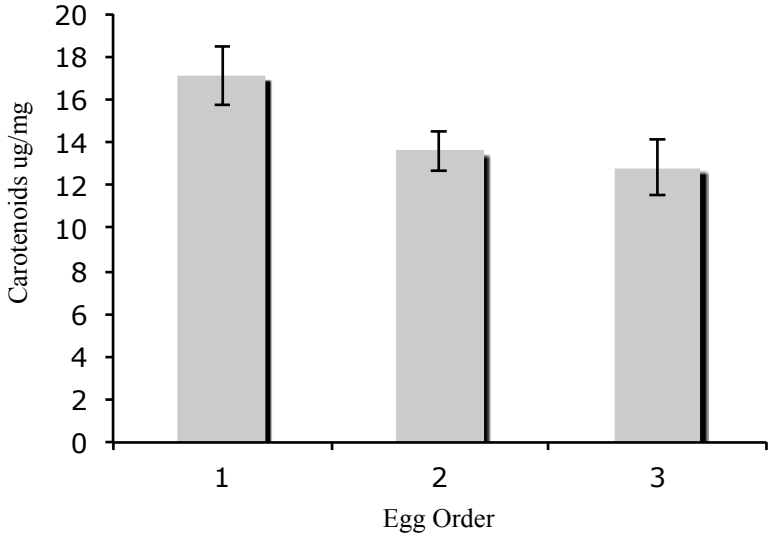

Figure 3. Egg Carotenoid Levels. Showing egg yolk carotenoid concentration based on laying order.

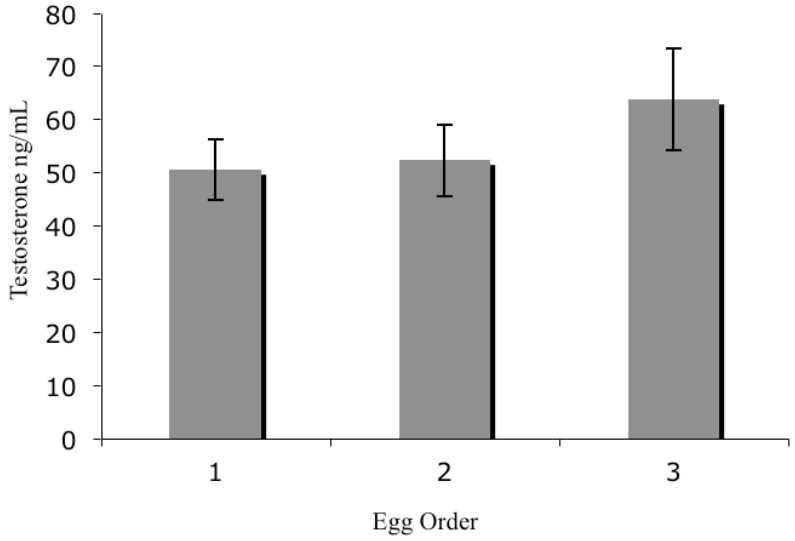

Figure 4. Egg Testosterone Levels. Showing egg testosterone concentration based on laying order.

Ornaments and Egg Components

Spearman rank correlation showed no relationship between carotenoid hue/brightness or saturation PC scores, with either average egg carotenoid content of eggs, or average egg T content of eggs (Table 4 and Figures 5, 6, 8 and 9). Additionally, melanin ornaments did not co-vary with and either T or carotenoid in egg yolks (Table 4 and Figures 7 and 10). Because there was no significant egg order effect on either carotenoids or T in the egg, only averages were used for correlations, and not individual eggs. Although there was no statistically significant correlation between egg components and ornamentation, there was a positive trend observed in the relationship between egg carotenoid levels and saturation PC score (Table 4 and Figure 6). Additionally, no relationship between average egg T and average egg carotenoids was found (Table 4 and Figure 11).

Table 4

Showing Spearman Rank Correlation Results Comparing Egg Carotenoid Levels, Egg T Levels, Hue/Brightness and Saturation and Mask Brightness

Variable	by Variable	R_S	P
Egg T	Egg Car	-0.087	0.7426
Hue/B	Egg Car	-0.1421	0.5017
Hub/B	Egg T	-0.1842	0.4503
Saturation	Egg Car	0.3982	0.0913
Saturation	Egg T	0.1211	0.6215
Saturation	Hue/B	-0.1632	0.5045
Melanin	Egg Car	-0.1526	0.5328Z
Melanin	Egg T	-0.1035	0.6733

* Denotes significant results.

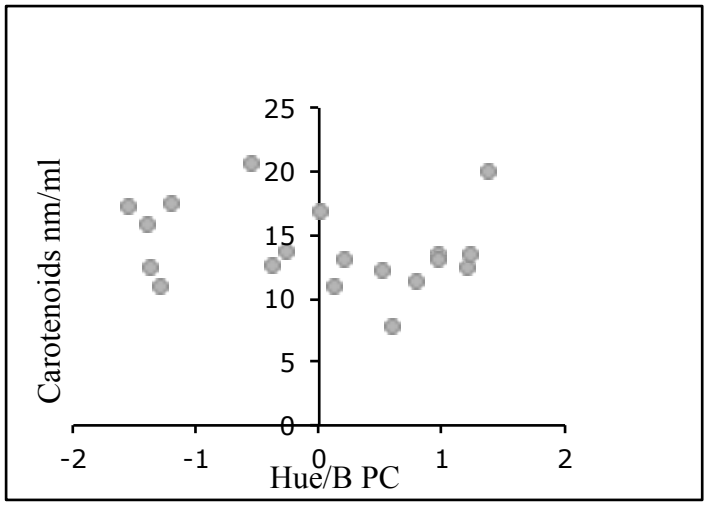

Figure 5. Hue/Brightness vs. Egg Carotenoid. Showing no relationship between carotenoid ornament Hue/B and carotenoid levels in the eggs.

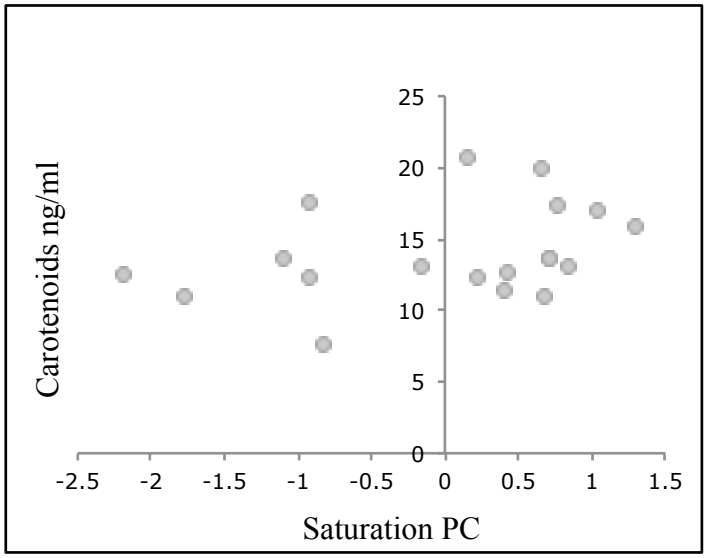

Figure 6. Saturation vs. Egg Carotenoid. Showing no relationship between carotenoid ornament saturation and carotenoid levels in the eggs.

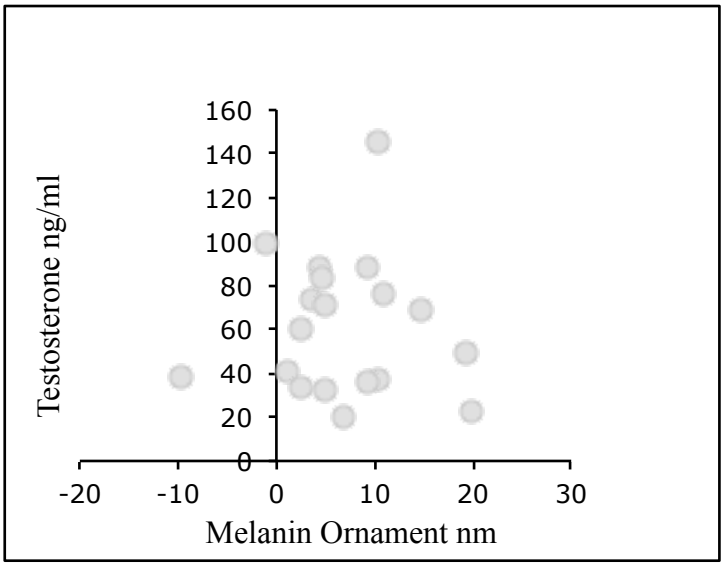

Figure 7. Melanin Ornament vs. Egg Testosterone. Showing no relationship between melanin ornament and egg testosterone.

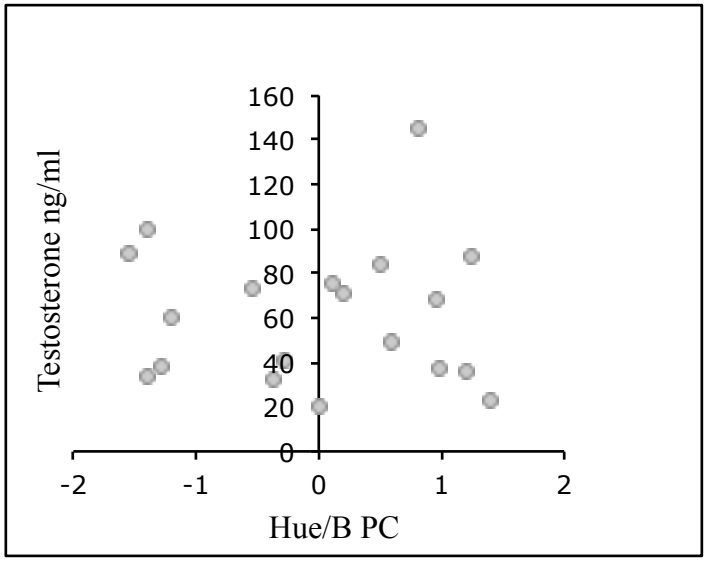

Figure 8. Hue/B vs. Egg Testosterone. Showing no relationship between carotenoid ornament Hue/B and egg testosterone.

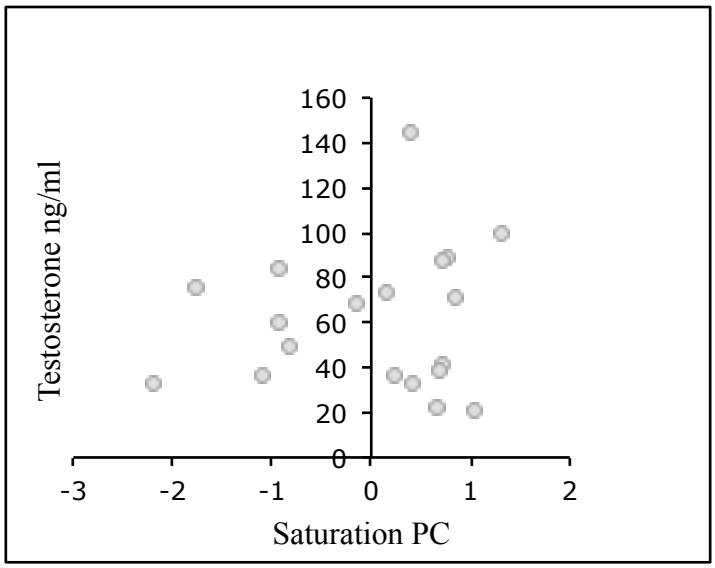

Figure 9. Saturation vs. Egg Testosterone. Showing no relationship between carotenoid ornament Saturation and egg testosterone.

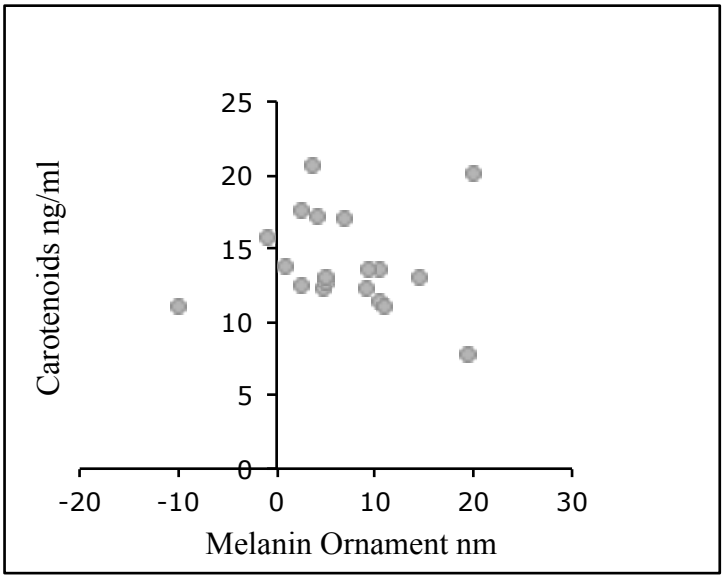

Figure 10. Melanin Ornament vs. Egg Carotenoid. Showing no relationship between melanin ornament and egg carotenoids.

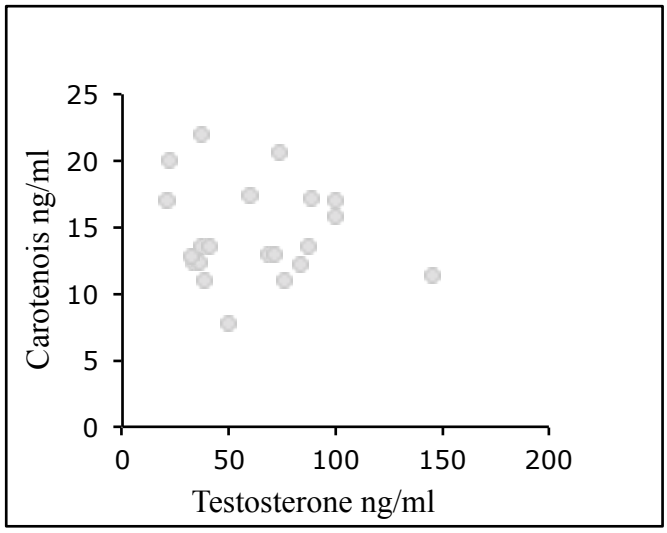

Figure 11. Egg Testosterone vs. Egg Carotenoid. Showing relationship between egg carotenoids and egg testosterone.

Conclusion

This study found no relationship between maternal effects (carotenoids or T) and either carotenoid or melanin ornamentation, as has been suggested in other species (Amundsen 2000). Therefore, results cannot provide evidence for The Good Parent Hypothesis as it refers to maternal effects in this species (Hoelzer 1989). Additionally, no relationship between carotenoids and T in the egg was found, although research suggests that this relationship may have compensatory effects on development (Royle et al. 2001; Török et al. 2007). We found no predictive pattern of T deposition in eggs. While explanations have been proposed to explain why female birds should have specific patterns of egg components, several studies seem to suggest that T deposition is not controlled, and is instead under more passive control (Schwabl 1996; Ellis et al. 2001; Whittingham and Schwabl 2002). Our results support this hypothesis, as no pattern was evident; however variation in average clutch T was dependent on the individual. Meaning that on average each female produced a predictable amount of T and individuals varied in how much T they placed in eggs. Conversely, a near significant pattern of deposition was discovered for carotenoids, with first eggs containing slightly more than the last two. While not significant, perhaps a higher sample size could elicit a pattern. Additionally, a near significant relationship exists between egg carotenoid levels and carotenoid ornament saturation. Saturation is usually regarded as quantity of carotenoids, and our results may indicate a trend that more saturated females are able to put more carotenoids into their eggs. Although not significant, there was variability between females with regard to carotenoid deposition. These females may have access to better

territories, or have greater liver health (McGraw 2006), as this is where carotenoid conversion takes place.

Ornamentation and Maternal Effects

Results do not support our hypotheses that ornamentation should predict egg-provisioning capabilities with respect to carotenoids or T. However we suggest that pairing history may play a role in the predictive nature of female ornaments for maternal effects. All females included in the study had been with their mate for at least one year, and ornamentation is thought to become less honest when birds pair for multiple seasons, and it appears that ornaments can become uncoupled from information about quality (including parental care) (Badyaev and Qvarnström 2002). In Southern portions of their range, female Northern Cardinals' greater expression of ornaments may be selected against, when the cost of production is higher than the informative benefit (Zahavi 1976). Research has supported that lesser expression may indicate allocation of resources to parental care, as opposed to self-maintenance, which has been demonstrated in several studies (Lemon et al. 1992; Qvarnström and Frosgren 1998; Griffith et al. 1999); a decrease in fitness of ornamented females may select for males that prefer less ornamented females (Griggio et al. 2009).

Egg Order

Literature suggests that females passively transfer androgens to egg yolks (Schwabl 1996). While it is unclear how patterns of T deposition occur, a lack of pattern seems to indicate plasticity in female circulating T during vitellogenesis (Whittingham and Schwabl 2002; Ellis et al. 2001). Female cardinals do indeed have circulating levels of testosterone during egg formation and laying (Jawor 2007). During the breeding

season females can elevate T in response to a chemically induced challenge (M.S. DeVries unpubl. data), whether this happens naturally during egg-laying is uncertain.

Additionally, it has been suggested that females differentially allocate greater concentrations of T to eggs that will yield males (as male offspring require more resources to grow; but also are potentially more profitable through increased reproductive potential), although the mechanisms behind this are unclear, it has been suggested that differing rates of follicle development in eggs which will become male or female (sex is determined by female in birds), may be a proximate mechanism for this (Pike and Petrie 2003). Because sex of the embryo was not evaluated in this study, it is unknown whether sex determination was responsible for variable levels of T within clutches.

This study indicates that ornaments should be addressed within the context of life-history stage, and past performance. Because Northern Cardinals are a monogamous, bi-parental species with pairs that stay together for multiple seasons, it is possible that their ornaments may no longer be informative after their initial pair formation (Badyaev and Qvarnström 2002). New pairs are known to mate assortatively based on ornamentation (Jawor et al. 2003) and new pairs should be assessed in order to determine which ornaments are more important during mate selection.

CHAPTER III
BEHAVIOR IN THE FEMALE NORTHERN CARDINAL

Introduction

Ornamentation is known to be indicative of aspects of quality and behavior in males of many species (Andersson 1994). As with ornamentation, males appear to be the showier sex with regard to aggression, and as such have received much attention among researchers (Wittenberger 1981). However, females are often aggressive as well (Yasukawa and Searcy 1982; Slagsvold 1993; Sandell and Smith 1997; Sandell 1998; Whittingham and Schwabl 2000) and for many of the same reasons as are described in males. In monogamous mating systems, females may benefit by using aggressive behavior to deter rival females from copulating with their partner (Slagsvold 1993; Sandell 1998), as males in these systems are a vital resource for raising young. Rival females may try and evict the current female, leading to a disastrous mate turnover for the primary female (Stephens 1982; Loftin and Robertson 1983; Hotta 1994). Additionally, females must protect their maternal investment by preventing other females from destroying their eggs or offspring or laying eggs in their nests (Petrie and Møller 1991), thereby providing a fitness benefit for both the male and the female. Clearly, although females are not aggressive to the same degree as males, they have very important reasons to behave so in the proper context. A female's capacity to defend resources aggressively is a quality that can be beneficial to advertise in many different social interactions. Therefore, this chapter addresses consequences of female aggression for both mates and rivals and uses the Northern Cardinal as a focal species.

Female Aggression and Ornamentation

In most species females do not defend territories throughout the year; this makes female cardinals, who do so, fairly unique among temperate birds. It has been suggested that female ornamentation may help males choose a female that can aid in territory defense (Amundsen 2000). In addition to defending her territory, females have also been shown to defend other resources; such as ensuring paternal care, through maintenance of monogamy. Studies have shown that female intrasexual aggression can play a role in social hierarchy of European Starlings (*Sturnus vulgaris*) (Sandell 1998), in which females attempt to maintain monogamy by deterring intruding females. Female Collared Flycatchers (*Ficedula albicollis*) have been shown to respond more aggressively to decoy intruders with greater ornament expression (Hegyi et al. 2008), indicating a motivation to defend against a more high quality female, which may be viewed as a greater threat. In Crested Auklets (*Aethia cristatella*), crest size has been shown to indicate level of intrasexual aggression in females, as well as been shown to be preferred by males (Jones and Hunter 1999); evidence that multiple receivers can benefit from information from a single ornament. In the Northern portions of their range, female Northern Cardinals have been shown to respond aggressively to simulated nest intrusions; females with darker melanin facemasks were shown to respond more aggressively (Jawor et al. 2004). Whether these associations remain true through Southern portions of their range is uncertain.

Study Species

As already mentioned, the melanin facemask of female Northern Cardinals has been shown to indicate intrasexual aggression in Northern portions of their range (Jawor

et al. 2004). Additionally, carotenoid ornaments have also been shown to be informative about condition and quality (Jawor et al. 2004). Therefore it is important to address both ornaments when considering informative content and implications for social behavior.

Cardinal ornamentation. Cardinal color ornamentation is based on two different pigment classes: carotenoids and melanins. Male ornamentation includes extensive red coloration on the body from food-derived carotenoid pigmentation, and a large black melanin-based facemask (Jawor et al. 2004). Females possess a smaller, and generally lighter facemask (although this ornament is highly variable in females), as well as red, carotenoid-based under-wing coverts, which are behaviorally displayed to males (Jawor and Breitwisch 2003; Jawor et al. 2004). Members of both sexes have an orange, carotenoid pigmented bill, as well as a tall head crest.

Carotenoids are considered to be condition dependent indicators of quality (Hill 1990). Carotenoid pigmentation must be obtained from the diet and reflects individual quality, but also availability of these compounds in the environment. While not closely associated with behavior, these ornaments have received attention for being honest indicators of quality through their condition dependence, therefore more high quality individuals may achieve greater expression by procuring resources (McGraw 2006). Melanins have recently received more attention for being honest indicators because they may have a genetic component, and expression is widely regarded to be testosterone-dependent (Jawor and Breitwisch 2003; Bókoney 2008). The expression of melanin ornaments has been shown to be associated with steroid hormones, and many steroid hormone receptors (including T) are known to exist on melanocytes; therefore they are very closely associated with behavior (Jawor and Breitwisch 2003). Melanin pigments

are produced by melanosomes in the skin from diet-obtained, non-essential amino acids, L-tyrosine or cystein (Jawor and Breitwisch 2003). The amino acids necessary to create melanin pigmentation may be food derived, but the ability to convert them into melanins is thought to be genetic (reviewed in Jawor and Breitwisch 2003). These two classes of pigments together may be capable of providing more accurate information about quality than each can alone. However, T-dependent traits are usually more closely associated with behavior than carotenoid-dependent traits.

Testosterone. As well as being behaviorally aggressive, female cardinals are also interesting because of their levels of measurable T year round (Jawor 2007). The connection between T and male aggression has been well studied, and although many females are often aggressive, the mechanics of hormonal control are not well known (Jawor et al. 2006). Research suggests that female cardinals have measurable T during egg laying (Jawor 2007). Although it is currently unclear if T is influencing aggressive behavior in female cardinals, if such a relationship existed a variety of phenotypic traits could also be influenced by this T, including ornamentation (melanin mask).

Because females of this species do possess multiple functional ornaments, the cardinal is a good model species to investigate several hypotheses concerning information conveyed by these ornaments. The use of this species is a rare chance to discover the relationship between ornaments and female aggression.

Signal Hypotheses

There is evidence that female Northern Cardinal's ornaments can be described by both the Multiple Message Hypothesis, and the Redundant Signal Hypothesis (Jawor and Breitwisch 2004). Female bill color and underwing redness have been shown to indicate

body size and aspects of condition. Underwing redness has also been shown to correlate with timing of the first nest, and reproductive success. However, facemask color and area have been shown to correlate with nestling feeding rate, and intrasexual aggression.

Given available evidence, I indirectly test female Northern Cardinal ornamentation under the Multiple Messages and Redundant Signal Hypotheses. Melanin ornaments have been shown to be indicative of intrasexual aggression in Northern populations (Jawor et al. 2004), and carotenoid ornaments have not. While carotenoid ornaments are not influenced by T, they are influenced by condition and quality. Therefore, carotenoid ornaments may reinforce information conveyed by T-influenced ornaments, and the Redundant Signal Hypothesis may be supported. If only one type of ornament indicates intrasexual aggression, this may indicate evidence for the Multiple Message Hypothesis, as the other ornament may indicate information about a different aspect of quality (such as maternal effects). Additionally, I wish to replicate a study done in a Northern population of non-captive cardinals, in which melanin ornaments were found to indicate level of aggression (Jawor et al. 2004) in a more Southern population to see if these findings hold when environmental conditions change.

Materials and Methods

This study was conducted over a two-year period from 2009-2010 at the Lake Thoreau Environmental Education and Research Center property owned by the University of Southern Mississippi (USM), a 131-acre site in Hattiesburg, Mississippi. Females to be included in the study were passively caught through the non-breeding and early breeding season with mist-nets and potter traps, and banded with a USFWS numbered stainless steel band, and three unique color bands for identification. During

the breeding season territories were identified by finding nests, which were usually occupied by a banded member of the population.

Behavioral Trials

A female's intrasexual aggressive response was determined by a Simulated Nest Intrusion (SNI) three days after completion of a clutch to ensure that the female did not abandon the nest due to human disturbance. A female decoy was placed at the nest of the incubating cardinal while the focal female was away from the nest to determine her aggressive response. The trial began once the female located the decoy, and lasted 5 minutes, or until she struck the decoy. Focal females were given a score from 1-5 based on her closest approach and whether or not she struck the decoy. One was given if she came back to the nest (all females came back to their nests, therefore the lowest possible score is a one), 2 was given if a female came <15 meters of the decoy, 3 was given if the female came <5 meters of the decoy, 4 was given if females came <5 meters of the decoy and displayed aggressively (including song, chipping, wing shivering or other types of physical displays), 5 was given if a female struck the decoy. Females were caught during these trials (for a different study), and ornament measures were taken if the female had not previously had ornaments assessed during the non-breeding season.

Ornaments

Either during passive capture or after nest intrusion, carotenoid and melanin ornaments of females were measured with an Ocean Optics USB4000 color reflectance spectrometer and a UV-VIS light source. For more detail on methods of quantification see Chapter II. In addition to melanin ornaments, crest length was recorded in mm (not thought to be functional, Jawor et al. 2004), and a mask score was determined. Mask

scoring included five categories, and took into account area and darkness, with five being the largest and darkest (see Figure 1). Melanin badge size was considered in this study, although size is thought to be under genetic control, rather than hormonal (Jawor and Breitwisch 2003).

Ornament Analysis

Spectral data were analyzed following the completion of fieldwork. Methods are detailed in Chapter II. Briefly for carotenoids hue, saturation, and brightness must be considered while for melanin coloration only brightness is considered, as it is the single most variable component of this coloration type.

Statistical Analysis

Statistical analyses were conducted in JMP 8 (2009 SAS Institute Inc.) for Spearman rank correlations, ANCOVA's and descriptive statistics (Table 5). For principal component analysis SPSS 9 (2009 SAS Institute Inc.) was used. Data were not normally distributed, but the tests used were considered robust enough to accommodate the data; when deemed appropriate, non-parametric tests were used (Spearman rank correlation). Because hue, saturation and brightness each explain a part of the color of an ornament, principal component analysis was used compress these three scores; hue and brightness were retained together, and saturation was not. Therefore, there are two scores to describe plumage coloration, a hue/brightness score and a saturation score. Although this study was conducted over two years, only ornaments of the year that females had a behavioral trial collected were used.

Results

Melanin Ornament and Behavior

Melanin mask brightness did not yield an association with SNI response (Figure 12), however the Spearman rank co-efficient suggests a positive trend, and this ornament appears to be highly variable (Table 5). Additionally, mask score (area) did not correlate with behavioral response (Table 6), and no relationship was found between behavioral response to the SNI and crest length (Table 6).

Table 5

Showing Descriptive Statistics for Ornamentation and Behavior

Variable	N	Mean	SD
Hue/Brightness	23	5.2632	±0.9999
Saturation	23	6.7826	±1.6034
Mask Melanin	23	6.7826	±6.9889
Mask Score (size)	23	2.4474	±0.8959
Crest	21	29.857	±2.1280
Behavioral Score	23	3.9333	±1.1629

Table 6

Showing Spearman Rank Correlations between Mask Melanin, Mask Score, Crest Length, and Behavioral Score

Variable	by Variable	R_s	P
Melanin	Mask Sore	-0.2143	0.4088
Melanin	Crest	0.1396	0.5688
Mask Score	Crest	0.1093	0.6559
Behavior	Melanin	0.3522	0.2168
Behavior	Mask Score	-0.1339	0.6626
Behavior	Crest	-0.0641	0.8205

*Denotes Significance

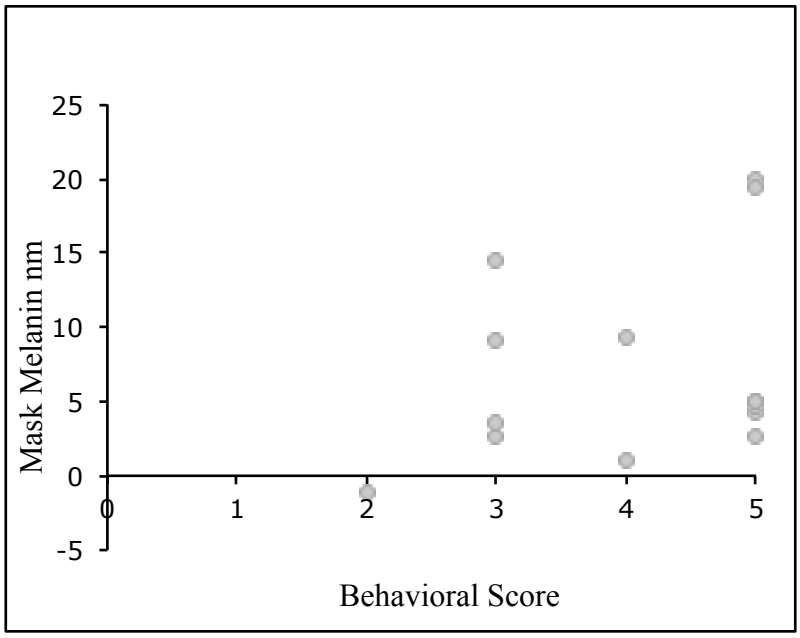

Figure 12. Mask Melanin vs. Behavioral Score. Showing slight positive non-significant trend between mask melanin and behavioral score.

Carotenoid Ornaments and Behavior

Hue/Brightness and Saturation did not show any relationship with the behavioral response (see Table 7 and Figures 13 and 14), or crest length (Table 7).

Table 7

Showing Spearman Rank Correlations between Hue/B, Saturation, Crest Length and Behavioral Score

Variable	by Variable	R_s	P
Hue/B	Behavior	0.1190	0.6853
Saturation	Behavior	-0.0452	0.8780
Crest	Hue/B	0.1298	0.5964
Crest	Saturation	0.0178	0.9424

*Denotes Significance

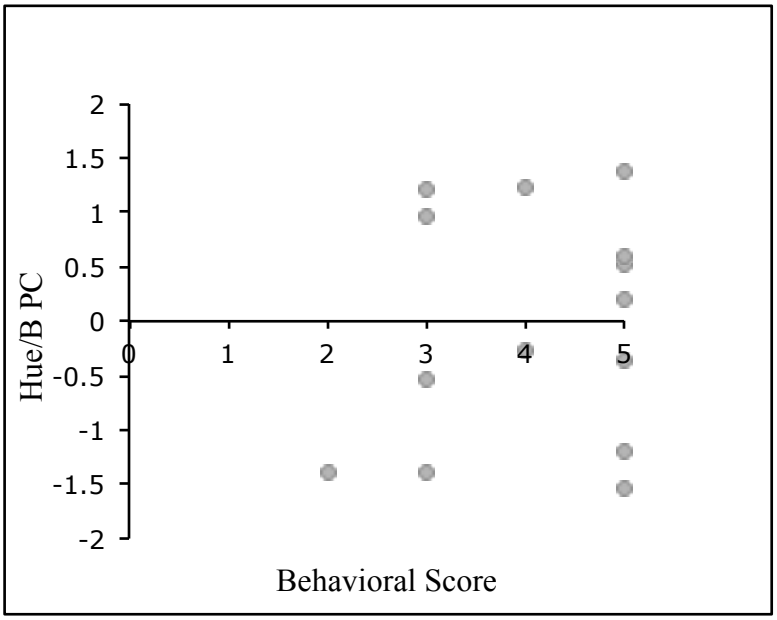

Figure 13. Hue/Brightness vs. Behavioral Score. Showing relationship between Hue/B and behavioral score.

Figure 14. Saturation vs. Behavioral Score. Showing relationship between Saturation and behavioral score.

Inter-Ornament Correlations

Although no behavioral data correlated with ornamentation, one intra-ornament correlation was significant; a strong correlation between melanin ornament and the Hue/B PCA was found (r_s = 0.8070 p = <0.0001, n = 23) Figure 15).

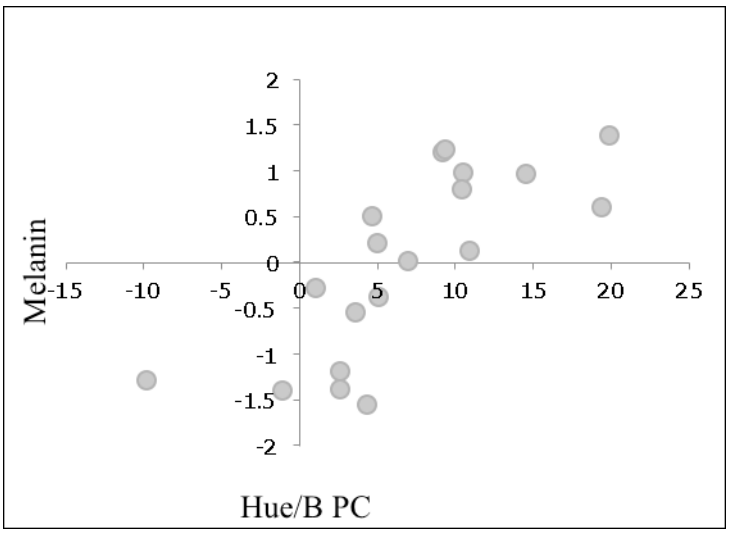

Figure 15. Mask Melanin vs. Hue/B. Showing the relationship between mask melanin and hue/B pc score.

Conclusion

Because there was no correlation between aggressive behavior score in females and melanin facemask or carotenoid ornament, it is uncertain what, if any, information is displayed by this ornament in intrasexual aggressive encounters in this population. Therefore, unlike in Northern populations, this study cannot provide evidence for the Multiple Messages Hypothesis, or the Redundant Signal Hypothesis (Møller and Pomiankowski 1993), as not only did melanin ornaments have no informative content in this context, but carotenoid ornaments do not appear to be informative of the aspects of quality investigated in this study either. While the traits assessed in this study were limited to intrasexual nest aggression, and therefore do not encompass all of the information important in mate selection and female-female competition, findings from this study do not corroborate evidence that female aggression is indicated by facemask in a more Northern population (Jawor et al. 2004). Therefore, this ornament may show a high level of variability between populations, perhaps resulting in selection for plasticity

in its expression and informative content in order to adjust to the different challenges this species faces in all portions of its range.

An unexpected result was the relationship between Hue/B and the melanin facemask. Generally, melanin ornaments are not considered to be condition dependent (but see, Jawor and Breitwisch 2003), and therefore should not necessarily co-vary with carotenoid ornaments. However, individuals of high genetic quality may be able to procure better territories and better mates, and carotenoid ornaments may reflect better condition as a result. Additionally, although melanins and carotenoids are often considered to be at opposite ends of the spectrum of condition dependence, expression of both are ultimately dependent on diet (even if to a lesser degree for melanins), and condition (Griffith 2006). It may be that carotenoid and melanin ornaments are redundant signals for some other aspect of quality not addressed in this study.

Intrasexual Aggression and Testosterone

Females in this study were assessed in an intrasexual context. However, female aggression is generally not well understood, and often must be evaluated on a case-by-case basis. It is not certain whether female cardinals respond to aggressive encounters with an increase in T at this time. Some studies have shown that females of other species do respond to naturally aggressive encounters with an increase in circulating T (Langmore et al. 2002; Mazuc et al. 2003; Gill 2007), however, others have not shown this relationship (Elekonich and Wingfield 2000; Hau et al. 2004; Jawor et al. 2006). While the hypothalamic-pituitary-gonadal axis of female Northern Cardinals can be stimulated to produce T during the breeding season; it is uncertain whether T is elevated during aggressive encounters specifically during egg laying (which is the time females

were assessed for behavior), or if T is even the female hormone of aggression in this species (M.S. DeVries unpubl. data). Although the Northern Cardinal is a temperate bird, its behavior profile is more similar to that of a tropical species. In the tropics both members of a pair defend the territory year-round (Thorpe 1972; Kunkel 1974; Farabaugh 1982; Dittami and Gwinner 1990), and have lower, but measurable circulating levels of T in both sexes year round (Gill et al. 2007). Low circulating levels of T may be supplemented with periodic elevations in T in aggressive contexts (Wingfield et al. 1990; Levin and Wingfield 1992; Goymann et al. 2004). However, in some tropical species aggression is not T dependent (Moore et al. 2004). If female Northern Cardinal aggression is not mediated by T, correlation between aggression and T-dependent melanin ornaments should not be expected. Whether T in the female Northern Cardinal plays a role in aggression, and the mechanisms behind the control of T production both still need to be determined. Although previous research does support a link between aggression and a melanin ornaments, how T influences aggression leaves the relationship between these two uncertain. Although female Northern Cardinals have measurable T, it is not safe to assume that this hormone facilitates aggressive behavior.

Signal Hypotheses

The findings of this study do not support the Multiple Messages Hypotheses, as this study did not find a message (within the aspects of behavior and quality addressed) conveyed by any ornament. However, the Unreliable Signal Hypothesis may be supported considering females reliably convey level of intrasexual aggression in one portion of their range, and not the other. The differences in the information displayed by the same ornament in two different populations may suggest a rapid evolution in the

informative content of this ornament. Differences in environment quality, climate, or genes (due to genetic drift) may select for differences in how ornaments are used to convey information. It is possible that in the Northern part of their range the breeding season for Northern Cardinals is more constrained by temperature (J.M. Jawor, pers. obs.). Melanin masks, which indicate aggression in Northern populations may be selected to do so as resources or time are limited, and research has shown birds that breed early in the season have greater reproductive success (Perrins 1970).

CHAPTER IV

DISCUSSION

In summary, our findings do not support the hypotheses presented for assessment. We did not find evidence that female ornaments may be used in accordance with the Good Parent Hypothesis when this hypothesis is extended to include maternal effects. Maternal effects known to be associated with ornamentation did not co-vary with female expression of carotenoid or melanin ornaments, as has been suggested might be the case (Amundsen 2000; Blount et al. 2002). Ongoing research investigating female testosterone production has shown that the female hypothalamic-pituitary-gonadal axis is active throughout egg laying and females can significantly elevate testosterone in cardinals and other species (see Jawor et al. 2007; M.S. DeVries, unpubl. data). This may obliterate any association between hormones being placed in eggs and hormones circulating at the time of ornament production. The same may be true of carotenoids given that female condition both at the time of ornament production and egg laying may significantly differ.

Nor did our findings provide evidence for the Redundant Signal or Multiple Messages Hypotheses, as neither type of ornamentation co-varied with intrasexually aggressive behavior, or maternal effects. This does not support a previous study from a different population of the same species, which found that female facemask co-varied with aggression (Jawor et al. 2004). Because neither carotenoid, nor melanin ornaments indicated an aspect of quality tested in this study, it is uncertain whether female cardinals' ornaments in this population follow the Multiple Message or Redundant Signal Hypotheses. The ornaments tested seem to be unreliable with regard to at least some

aspects of quality. Therefore, this research supports the Unreliable Signal Hypothesis, as ornaments in female Northern Cardinals do not appear to be an honest indicator of the qualities assessed in this population, as they are in others, and suggests that different populations may use ornaments in communication in different fashions. Future studies on other widely spread species may find similar changes in ornament signal use. However, Hue/B and mask melanin were highly correlated, suggesting that these ornaments may indeed be reliable signals of aspects of quality not addressed in this study. The information and honesty of ornaments can vary according to condition and life-history stage, which varies with time and geographic location, and this should be considered when addressing ornaments of different populations (Badyaev and Qvarnström 2002).

Multiple Receivers Hypothesis

Our results do not provide evidence for the Multiple Receivers Hypothesis. While females in a population of Northern Cardinals in a more Northern part of the species range convey ability to respond aggressively in intrasexual encounters, these findings were not corroborated in a Southern population. Female cardinal ornaments do not appear to be informative in an intrasexual context in this population, do they appear to be informative as indicators of parental care (an extension of the Good Parent Hypothesis) (Hoelzer 1989). Therefore, while it has been suggested that female cardinal melanin ornaments may demonstrate messages to multiple receivers (Jawor et al. 2004), evidence from this study cannot support that hypothesis.

Egg Testosterone

It has been suggested that females who respond aggressively during egg-laying passively transmit more T to egg yolks during vitellogenesis (Schwabl 1996; Whittingham and Schwabl 2000; Mazuc et al. 2003). Although female cardinals with greater expression of melanin ornaments respond more aggressively in Northern portions of their range, it is not clear that T was involved in facilitating this behavior. Although, it is possible that female cardinals are capable of altering T concentration in plasma to facilitating aggressive behavior for protection of resources (M.S. DeVries, unpubl. data), it is not clear that this T is being deposited in eggs. However, we did not experimentally manipulate females to be aggressive at the nest that was collected. Therefore, we can only assume that females capable of behaving aggressively do so at each nest attempt. A more experimental approach may be required to test this hypothesis in the Northern Cardinal. It is also possible that female circulating T levels during egg-laying may be constrained to protect eggs from harmful levels of T (Møller et al. 2005). Hazardous levels of T can not only kill embryos, but can also create long-term health disadvantages, such as decreased immunity (Andersson et al. 2004), and trade-offs in growth and behavior (Møller et al. 2005).

Egg Carotenoids

While our study did not find a relationship between egg carotenoids and any ornamentation in female cardinals, there was a near significant difference in first eggs. Various research supports patterns of decreasing order (Saino et al. 2002), increasing order (Royle et al. 2001), and differential carotenoid deposition for each sex (McGraw et al. 2005; Badyaev et al. 2006). Physiological mechanisms for carotenoid deposition are

not well understood (Blount et al. 2002), however, a prospective mechanism for decreasing order is that carotenoid reserves in plasma may build up prior to laying (as food becomes readily available), and then become depleted over the course of laying (Saino et al. 2002). Additionally, no relationship between T and carotenoids was found in eggs, as has been indicated in previous research (Török et al. 2007).

Honesty in Ornamentation

For an ornament to be reliable, it must be costly to produce or maintain (Zahavi 1975). Melanins are more commonly assumed to be indicators of genetic quality (Jawor and Breitwisch 2003) than carotenoids. It is thought that because expression of melanin ornaments is dependent on steroid hormone receptors on melanocytes, and steroid hormone production is genetically linked, that "cheaters" are rare, as only high quality individuals should be able to incur the cost of maintaining high T (Jawor and Breitwisch 2003). Individuals that have higher T are often more aggressive and the hormone link between behavior and ornament expression help maintain the honesty of this ornament. However, the findings from this study do not support this hypothesis; although it is possible that the ornaments assessed are, indeed, honest indicators of qualities not assessed by this study, and an investigation of steroid hormones and melanin ornaments will need to be completed in this species.

Additionally, "quality" is a very broad term, and it can change from day to day (Jawor and Breitwisch 2003). Therefore, it is possible that female's assessed for behavior were not capable of backing up their ornament on the particular day assessed but could do so at another time. Carotenoids are well known to be condition-dependent indicators of quality (Hill 1990); therefore, it may not be surprising that as eggs are

yolked over a relatively short time scale, illness, drought, or other natural disasters may reduce a female's ability to provision eggs for one nest, even if she is typically capable, thus making the link between an ornament and maternal effects at any given point more tenuous.

Ornaments are often viewed as static, but birds have ornaments that are very dynamic; the Northern Cardinal's plumage ornaments change once a year during molt. That being said, ornaments in the fall may not be informative during the spring. Ornaments have been shown to become uncoupled from the qualities they should indicate over time and particularly in long-term pair bond species like the cardinal (Zuk and Johnsen 1998; Gonzalez et al. 1999). Additional research will be needed to fully understand how female ornaments are used in Northern Cardinals to convey information on behavior and condition in Southern populations.

Life-History Perspective on Ornamentation

It is important to remember that ornaments display information that should be relevant within certain contexts. Life history trade-offs and strategies should be considered when evaluating expression and function of such ornaments (Badyaev and Qvarnström 2002). In cases where expression of an ornament imposes an unnecessary constraint on the reproductive fitness of an organism, the individual is at a disadvantage, as opposed to an advantage, by investing in an ornament as opposed to other costly traits and behaviors (Andersson 1982, Partridge 1987, Badyaev and Hill 1999).

When an ornament is expressed, and to what degree the expression occurs can be expected to be variable in species that remain paired for multiple seasons, and would therefore need to maintain costly ornaments for a longer time period than short-term

breeders (Badyaev and Qvarnström 2002). Research is emerging which suggests that birds, which have the opportunity to change their sexually selected ornaments seasonally through molt, benefit from the opportunity to adjust ornament expression depending on condition and life-history stage (Badyaev and Duckworth 2003). In House Sparrows (*Passer domesticus*), less ornamented males are preferred and had greater reproductive success (Griffith et al. 1999). Ornaments created during first adult molt were correlated with condition, however, they became independent of condition or quality as the bird aged (see Badyaev and Duckworth 2003). Pairing status in the most recent breeding season prior to molt was found to be the best indicator of reproductive success, not ornament expression. This should allow individuals to allocate resources appropriately for their current life-history stage. This study indicates that ornaments are highly context dependent, and the ability to maximize flexibility in ornament expression may be selected for in this species. Often, male and females with less expression of ornaments have been found to be better parents, although in one population of Northern Cardinals this was not the case (Linville et al. 1998). Also, although female Northern Cardinals have ornaments that have been shown to display information about quality, mate choice experiments have not been performed. Although female ornaments were originally thought to be non-functional (Lande 1980), many studies now support functional hypotheses for female ornamentation (Amundsen and Pärn 2006). However, it can only be assumed that males assess female quality through ornaments in this species.

Geographical Variation

In wide-ranging species, it seems unreasonable that the same information should be of equal relevance throughout the range of a single species (Badyaev and Qvarnström

2002). Environmental differences can reveal varying preference for traits in mates due to differences in ability to maintain condition. Ornament variation has been seen in House Finches (Hill 1993; Hill 1994), Barn Swallows (*Hirundo rustica*) (Møller 1995), and Common Yellowthroats (*Geothlypis trichas*) (Dunn et al. 2008). Additionally, mate preference can vary with geographic location (perhaps driving selection), (Endler and Houde 1995; Hill 1994; Kwiatkowski and Sullivan 2002). Changes in preference may be based on differences in food availability, or climate (Grether 2000). When a species migrates to a new location, ornaments that were once functional in a different environment may fall under the Unreliable Signal Hypothesis; they are maintained genetically but are now not relevant to mate choice in the new area. This has been demonstrated in House Sparrows (Griffith et al. 1999), Norwegian Pied Flycatchers (*Ficedula hypoleuca*) (Dale et al. 1999), and Red-winged Blackbirds (*Agelaius phoeniceus*) (Westneat 2006).

This study failed to find a relationship between melanin ornamentation in female Northern Cardinals and aggressive response and maternal effects. These results indicate that this ornament may be highly variable between populations, and the potential relationship T plays in behavior and melanin expression should be addressed on a case-by-case basis for this species. This population in a Southern part of the Northern Cardinal's range may provide evidence for the Unreliable Signal Hypothesis (Møller and Pominankowski 1993). There are several areas of this study in which further investigation may yield a better understanding of the proximate and ultimate mechanisms of maternal effects, ornamentation and behavior in female Northern Cardinals. The trend of more highly carotenoid ornamented females producing eggs with higher

concentrations of carotenoids in them should be investigated further, as the near significant results from this study are inconclusive. Additionally, mate choice experiments are needed to determine whether female ornaments are under the selective pressure of male-choice, or female-female intrasexual aggression. The relationship between egg order and T deposition revealed no pattern, and therefore provides evidence that maternal T to yolk transfer may be under more passive control than has been suggested for other species. The function of no control over T deposition in eggs should be investigated further. Carotenoid deposition in eggs was nearly significant, and larger sample sizes may indeed reveal a pattern. The hormone that facilitates female aggressive behavior in this species is thought to be T, and although females do have measureable T during the breeding season, and are known to behave aggressively during the breeding season, more direct experimental evidence is needed to provide support for this theory.

APPENDIX

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE APPROVAL FORM

The University of Southern Mississippi

Institutional Animal Care and Use Committee

118 College Drive #5147
Hattiesburg, MS 39406-0001
Tel: 601.266.6820
Fax: 601.266.5509
www.usm.edu/spa/policies/animals

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
NOTICE OF COMMITTEE ACTION

The proposal noted below was reviewed and approved by The University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC) in accordance with regulations by the United States Department of Agriculture and the Public Health Service Office of Laboratory Animal Welfare. The project expiration date is noted below. If for some reason the project is not completed by the end of the three year approval period, your protocol must be reactivated (a new protocol must be submitted and approved) before further work involving the use of animals can be done.

Any significant changes (see attached) should be brought to the attention of the committee at the earliest possible time. If you should have any questions, please contact me.

PROTOCOL NUMBER: **08081401**
PROJECT TITLE: **Female Ornaments and Behavior, Offspring Quality, and Male Mate Choice in a Dimorphic Songbird**
PROPOSED PROJECT DATES: **01/01/2009 to 12/31/2011**
PROJECT TYPE: **Renewal of a Previously Approved Project**
PRINCIPAL INVESTIGATOR(S): **Jodie Jawor, Ph.D.**
COLLEGE/DIVISION: **College of Science & Technology**
DEPARTMENT: **Biological Sciences**
FUNDING AGENCY/SPONSOR: **N/A**
IACUC COMMITTEE ACTION: **Full Committee Review Approval**
PROTOCOL EXPIRATION DATE: **09/30/2011**

Robert C. Bateman, Jr., Ph.D.
IACUC Chair

August 14, 2008
Date

WORKS CITED

Adkins, E.K. 1975. Sex Steroids and the differentiations of avian reproductive behavior. American Zoologist. 18: 501-509.

Adkins, E.K. 1977 Effects of diverse androgens on the sexual behavior and morphology of castrated male quail. Hormones and Behavior. 8: 201-207.

Adkins-Regan, E. 1987. Sexual differentiation in birds. Trends in Neurosciences. 10: 517-522.

Adkins-Regan, E. 2005. Hormones and Animal Social Behavior. Princeton University Press, Princeton, New Jersey.

Amundsen, T. 2000. Why are female birds ornamented? Trends in Ecology and Evolution. 15: 149-155.

Amundsen, T., Forsgren, E. & Hansen, L.T.T. 1997. On the function of female ornaments: male bluethroats prefer colourful females. Proceedings of the Royal Society of London, Series B. 264: 1579-1586.

Amundsen, T. & Pärn, 2006. Female Coloration: Review of Functional and Non-Functional Hypotheses. Bird Coloration: Function and Evolution. Harvard University Press, Cambridge, MA.

Andersson, M. 1982. Female choice selects for extreme tail length in a widowbird. Nature. 299: 818-820.

Andersson, M. 1986. Evolution of condition-dependent sex ornaments and mating preferences: sexual selection based on viability differences. Evolution. 40: 804-816.

Andersson, M. 1994. Sexual Selection. Princeton University Press, Princeton, New Jersey.

Andersson, S., Pryke, S.R., Örnborg, J., Lawes, M.J., Andersson, M. 2002. Multiple receivers, multiple ornaments, and a trade-off between agnostic and epigamic signaling in a widowbird. The American Naturalist. 160: 683-691.

Andersson, S., Uller, T., Löhmus, F. 2004. Effects of egg yolk testosterone on growth and immunity in a precocial bird. Journal of Evolutionary Biology. 17: 501-505.

Badyaev, A.V. & Hill, G.E. 1999. Variation in avian sexual dimorphism in relation to phylogeny and ecology: a review. Proceedings of the XXII International Ornithological Congress. 1687-1709.

Badyaev, A.V. & Qvarnstrom, A. 2002. Putting sexual traits into the context of an organism: a life-history perspective in studies of sexual selection. The Auk. 119: 301-310.

Badyaev, A.V. & Duckworth, R.A. 2003. Context-dependent sexual advertisement: plasticity in development of sexual ornamentation throughout the lifetime of a passerine bird. Journal of Evolutionary Biology. 16: 1065-1076.

Badyaev, A.V., Seaman, D.A., Navara, K.J., Hill, G.E., Mendonça, M.T. 2006. Evolution of sex-biased maternal effects in birds: III. Adjustments of order can enable sex-specific allocation of hormones, carotenoids, and vitamins. Journal of Evolutionary Biology. 19: 1044-1057.

Balthazrt, J., Ball, G.F. 1995. Sexual differentiation of brain and behavior in birds. Trends in Endocrinology and Metabolism. 6: 21-29.

Biard, C., Gill, D., Karadaş, F., Saino, N., Spottiswoode, C.N., Surai, P.F., Møller, A.P. 2009. Maternal effects mediated by antioxidants and the evolution of carotenoid-based signals in birds. The American Naturalist. 174: 696-708.

Blas, J., Pérez-Rodríguez, L., Bortolotti, G.R., Viñuela, J., Marchant, T.A. 2006. Testosterone increases bioavailability of carotenoids: insights into the honest of sexual signaling. Proceedings of the National Academy of Sciences of the United States of America. 103: 18633-18637.

Blount, J. D., Houston, D.C., Møller, A.P. 2000. Why egg yolk is yellow? Trends in Ecology and Evolution. 15: 47-49.

Blount, J. D., Surai, P.F., Nager, R.G., Houston, D.C., Møller, A.P., Trewby, M.L. & Kennedy, M.W. 2002. Carotenoids and egg quality in the lesser black-backed gull *Larus fuscus*: a supplemental feeding study of maternal effects. Proceedings of the Royal Society of London. 269: 29-36.

Bokony, V., Garmszegi, L.Z., Hirschenhauser, K., Liker, A. 2008. Testosterone and melanin-based black plumage coloration: a comparative study. Behavioral Ecology and Sociobiology. 62: 1229-1238.

Chenoweth, S.F., Doughty, P., Kokko, H. 2006. Can non-directional male mating preferences facilitate honest female ornamentation? Ecology Letters. 9: 179-184.

Chew, B.P. 1996. Importance of antioxidant vitamins in immunity and health in animals. Animal Feed Science Technology. 59: 103-114.

Dale, S., Slagsvold, T., Lampe, H.M., Sætre, G.P. 1999. Population divergence in sexual ornaments: the white forehead patch of Norwegian pied flycatchers is small and unsexy. Evolution. 53: 1235-1246.

Darwin, C. 1871. The Descent of Man, and Selection in Relation to Sex. Murray, London.

Doutrelant, C., Grégorie, A., Grnac, N., Gomez, D., Lambrechts, M.M., Perret, P. 2008. Female coloration indicates female reproductive capacity in blue tits. Journal of Evolutionary Biology. 21: 226-233.

Dunn, P.O., Whittingham, L.A., Freeman-Gallant, C.R., DeCoste, J. 2008. Geographic variation in the function of ornaments in the common yellowthroat (*Geothlypis trichas*). Journal of Avian Biology. 39: 66-72.

Eising, C.M., Eikenaar, C., Schwabl, H., Groothuis, T.G.G. 2001. Maternal androgens in black-headed gull (*Larus ridibundus*) eggs: consequences for chick development. Proceedings of the Royal Society of London. 268: 839-846.

Elekonich, M.M. & Wingfield, J.C. 2000. Seasonality and hormonal control of territorial aggression in female song sparrows (Passeriformes: Emberizidae: *Melospiza melodia*). Ethology. 106: 493-510.

Ellis, L.A., Borst, D.W., Thompson, C.F. 2001. Hatching asynchrony and maternal androgens in egg yolks of house wrens. Journal of Avian Biology. 32: 26-30.

Enderl, J.A. & Houde, A.E. 1995. Geographic variation in female preference for male traits in *Poecilia reticulate*. Evolution. 49: 456-468.

Farabaugh, S.M. 1982. The Ecological and Social Significance of Duetting. Pages 85-124 *in* Acoustic Communication in Birds, vol.2 (D. S. Kroodsma and E. H. Miller, Eds.). Academic Press, New York.

Fischer, R. A. 1930. The Genetical Theory of Natural Selection. Clarendon Press, Oxford.

Gil, D. 2003. Golden eggs: maternal manipulation of offspring phenotype by egg androgen in birds. Ardeola. 50: 281-294.

Gill, S.A., Alfson, E.D., Hau, M. 2007. Context matters: female aggression and testosterone in a year-round territorial neotropical songbird (*Thyrothorus leucotis*). Proceeding of the Royal Society of London. 274: 2187-2194.

Gonzalez, G., Sorci, G., de Lope, F. 1999. Seasonal variation in the relationship between cellular immune response and badge size in male house sparrows (*Passer domesticus*). Behavioral Ecology and Sociobiology. 46: 117-122.

Goodwin, T.W. 1984. The Biochemistry of the Carotenoids. Chapman and Hall, New York.

Goymann, W. Moore, I.T., Scheuerlein, A., Hirschenhauser, K., Grafen, A., Wingfield, J.C. 2004. Testosterone in tropical birds: effects of the environment and social factors. The American Naturalist. 164: 327-334.

Grether, G.F. 2000. Carotenoid limitation and mate preference evolution: a test of the indicator hypothesis in guppies (*Poecilia retriculata*). *Evolution*. 54: 1712-1724.

Griffith, S.C., Owens, I.P.F., Burke, T. 1999. Female choice and annual reproductive success faour less-ornamented male house sparrows. . Proceeding of the Royal Society of London, Series B. 266: 765-770.

Griffith, S.C., Parker, T.H., Olson, V.A. 2006. Melanin-verses carotenoid-based sexual signals: is the difference really so black and red? Animal Behaviour. 71: 749-763.

Griggio, M., Valera, F., Casas, A. & Pilastro, A. 2005. Males prefer ornamented females: a field experiment of male choice in the rock sparrow. Animal Behaviour. 69: 1243-1250.

Griggio, M., Devigili, A., Hoi, H., Pilastro, A. 2009. Female ornamentation and directional male mate preference in the rock sparrow. Behavioral Ecology. 20: 1072-1078.

Groothuis, T.G., Eising, C. M., Dijkstra, C. & Müller, W. 2005. Balancing between costs and benefits of maternal hormone deposition in avian eggs. Proceeding of the Royal Society of London, Biology Letters. 1: 78-81.

Gwinner, E. & Dittami, J. 1990. Endogenous reproductive rhythms. *Science* 249: 906-908.

Halkin, S.L. & Linville, S.U. 1999. Northern Cardinal (*Cardinalis cardinalis*). Pages 1-32 *in* Birds of North America, no. 440 (A. Poole and F. Gill, Eds.). Birds of North America Inc., Philadelphia, Pennsylvania.

Hamilton, W.D. & Zuk, M. 1982. Heritable true fitness and bright birds: a role for parasites? Science. 218: 384-387.

Hanssen, S.A., Folstad, I., Erikstad, K.E. 2006. White plumage reflects individual quality in female eiders. Animal Behaviour. 71: 337-343.

Hargitai, R., Török, J., Tóth, L., Hegyi, G., Rosivall, B., Szigeti, B., Szöllősi. 2005. Effects of environmental condition and parental quality on inter- and intraclutch egg-size variation in the collard flycatcher (*Ficedula albicollis*). The Auk. 122: 509-522.

Hau, M., Stoddard, S.T., Soma, K.K. 2004. Territorial aggression and hormones during the non-breeding season in a tropical bird. Hormones and Behavior. 45: 40-49.

Hegyi, G., Garamszegi, L., Z., Eens, M., Török, J. 2008. Female ornamentation and territorial conflicts in collared flycatchers (*Ficedula albicollis)*. Naturwissenschaften. 95: 993-996.

Hill, G.E. & Montgomerie, R. 1990. Plumage colour signals nutritional condition in the house finch. Proceedings of the Royal Society of London. 258: 47-52.

Hill, G.E. 1993. Geographic variation in the carotenoid plumage pigmentation of male house finches (*Carpodacus mexicanus*). Biological Journal of the Linnean Society. 49: 63-86.

Hill, G.E. 1993. Geographic variation in male ornamentation and female mate preference n the house finch: a comparative test of models of sexual selection. Behavioral Ecology. 5: 64-73.

Hoelzer, G.A. 1989. The good parent process of sexual selection. Animal Behaviour. 38: 1067-1078.

Hotta, M.N. 1994. Infantacide in little swifts taking over costly nests. Animal Behaviour. 47: 491-493.

Hutchinson, R.E. 1978. Hormonal Differentiation of sexual behavior in Japanese quail. Hormones and Behavior. 11: 363-387.

Jawor, J.M. 2007. Testosterone in Northern cardinals (*Cardinalis cardinalis*) possible influence of prolonged territorial behavior. The Auk. 124: 331-338.

Jawor, J. M. & Breitwisch, R. 2003. Melanin ornaments, honesty, and sexual selection. The Auk. 120: 249-265.

Jawor, J.M. & Breitwisch, R. 2004. Multiple ornaments in male northern cardinals, *Cardinalis, cardinalis*, as indicators of condition. Ethology. 110: 113-126.

Jawor, J.M., Linville, S.U., Beall, S.M, Breitwisch, R. 2003. Assortative mating by multiple ornaments in northern cardinals (*Cardinalis cardinalis*). Behavioral Ecology. 14: 515-520.

Jawor, J.M., Gray, N., Beall, S. & Breitwisch, R. 2004. Multiple ornaments correlate with aspects of condition and behavior in female northern cardinals, *Cardinalis cardinalis.* Animal Behaviour. 67: 875-882.

Jawor, J.M., Young, R., Ketterson, E.D. 2006. Female competing to reproduce: dominance matters, but testosterone may not. Hormones and Behavior. 49: 362-368.

Jones, I. L. & Hunter, F. M. 1993. Mutual sexual selection in a monogamous seabird. Nature. 362: 238-239.

Jones, I. L. & Hunter, F. M. 1999. Experimental evidence for mutual inter- and intrasexual selection favouring a crested auklet ornament. Animal Behaviour. 57: 521-528.

Komdeur, J., Oorebeek, M., van Overveld, T., Cuthill, I.C. 2005. Mutual ornamentation, age, and reproductive performance in the european starling. Behavioral Ecology. 16: 805-817.

Kunkel, P. 1974. Mating systems of tropical birds: the effect of weakness or absence of external reproduction-timing factors, with special reference to prolonged pair bonds. Zeitschrift für Tierpsychologie. 34: 265-307.

Kwiatkowski, M.A. & Sullivan, B.K. 2002. Geographic variation in sexual selection among populations of an iguanid liazard, *Sauromalus obesus*. Evolution. 56: 2039-2051.

LaBas, N.R., Hockham, L.R., Ritchie, M. 2003. Nonlinear and correlational sexual selection on "honest" female ornamentation. Proceedings of the Royal Society of London. 270: 2159-2165.

Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution. 34: 292-305.

Langmore, N.E., Davies, N.B., Hatchwell, B.J. 1996. Female song attracts males in the alpine accentor (*Prunella collaris*). Proceedings of the Royal Society of London. 263: 141-146.

Langmore, N.E., Cockren, J.F., Candy, E.J. 2002. Competition for male reproductive investment elevates testosterone levels in female dunnocks (*Prunella modularis*). Proceedings of the Royal Society of London. 269: 2473-2478.

Lemon, R.E., Weary, D.M., Norris, K.J. 1992. Male morphology and behavior correlates with reproductive success in the American redstart (*Setophaga ruticilla*). Behavioral Ecology and Sociobiology. 29: 399-403.

Levin, R.N. & Wingfield, J.C. 1992. Hormonal control of territorial aggression in tropical birds. Ornis Scandinavica. 23: 284-291.

Linville, S.U., Breitwisch, R., Schilling, A.J. 1998. Plumage brightness as an indicator of parental care in northern cardinals. Animal Behavior. 55: 119-127.

Lipar, J. L. & Ketterson. E.D. 2000. Maternally derived yolk testosterone enhances the development of the hatching muscle in the red-winged blackbird *Agelaius phoeniceus*. Proceedings of the Royal Society of London. 267: 2005-2010.

Lipar, J.L., Ketterson, E.D., Nolan, Jr., Val. 1999. Intraclutch variation in testosterone content of red-winged blackbird eggs. The Auk. 116: 231-235.

Loftin, R.W. & Robertson, D. 1983. Infantacide by a purple martin. Wilson's Bulletin. 95: 146-148.

Massironi, M., Rasotto, M.B., Mazzoldi, C. 2005. A reliable indicator of female fecundity: the case of the yellow belly in *Knipowitschia panizzae* (Teleostei: Gobiidae). Marine Biology. 147: 71-76.

Mazuc, J., Bonneaud, C., Chastel, O., Sorci, G. 2003. Social environment affects female and egg testosterone levels in the house sparrow (*Passer domesticus*). Ecology Letters. 6: 1084-1090.

McGraw, K.J. 2006. Mechanics of Melanin-Based Coloration. Pages 243-294 *in* Bird Coloration: Mechanisms and Measurements, vol. 1 (G.E. Hill and K.J. McGraw Eds.). Harvard University Press, Cambridge, MA.

McGraw, K.J. 2006. Mechanics of Carotenoid-Based Coloration. Pages 177-242 *in* Bird Coloration: Mechanisms and Measurements, vol. 1 (G.E. Hill and K.J. McGraw Eds.). Harvard University Press, Cambridge, MA.

McGraw, K.J. & Hill, G.E. 2000. Differential effects of endoparasitism on the expression of carotenoid- and melanin-based ornamental coloration. Proceedings of the Royal Society of London. 267: 1525-1531.

McGraw, K.J., Adkins-Regan, E., Parker, R.S. 2005. Maternally derived carotenoid pigments affect offspring survival, sex ratio, and sexual attractiveness in a colorful songbird. Naturwissenschaften. 92: 375-380.

Møller, A.P. & Pomiankowski, A. 1993. Why have birds got multiple sexual ornaments? Behavioral Ecology and Sociobiology. 31: 167-176.

Møller, A.P., Garamszegi., L.Z., Gil, D., Hurtrez-Boussés, S., Eens, M. 2005. Correlated

evolution of male and female testosterone profiles in birds, and it's consequences. Behavioral Ecology and Sociobiology. 58: 534-544.

Moore, I.T., Walker, B.G., Wingfield, J.C. 2004. The effects of combined aromatase inhibitor and anti-androgen on male territorial aggression in a tropical population of rufous-collared sparrows, (*Zonotrichia capensis*). General and Comparative Endocrinology. 135: 223-229.

Mosseau, T.A. & Fox, C.W. 1998. Adaptive significance of maternal effects. Trends in Ecology & Evolution. 13: 403-407.

Murphy, T.G. 2006. Racketed tail of the male and female turquoise-browed motmot: male but not female tail length correlates with pairing success, performance, and reproductive success. Behavioral Ecology and Sociobiology. 61: 911-918.

Murphy,T.G., Hernández-Muciño, D., Osorio-Beristain, M., Montgomerie, R. & Omland K.E. 2009. Carotenoid-based status signaling by females in the tropical streak-backed oriole. Behavioral Ecology. 20: 1000–1006

Murphy, T. G., Rosenthal, M. F., Montgomerie, R., & Tarvin, K. A. 2009. Female American goldfinches use carotenoid-based bill coloration to signal status. Behavioral Ecology. 20: 1348-1355.

Navara, K.J., Hill, G.E., Mendonça, M.T. 2006. Yolk testosterone stimulates growth and immunity in house finch chicks. Physiological and Biochemical Zoology. 79: 550-555.

Owens, I.P. & Thompson, D.B.A. 1994. Sex differences, sex ratios and sex roles. Proceedings of the Royal Society of London. 258: 93-99.

Parker, G.A. 1983. Mate Quality and Mating Decisions. Pages 141-166 *in* Mate Choice (P. Bateson Ed.). Cambridge University Press, New York.

Parker, T.H. 2002. Maternal condition, reproductive investment, and offspring sex ratio in captive red junglefowl (*Gallus gallus*). The Auk. 119: 840-845.

Partridge, L. 1987. Life-history constrains on sexual selection. Pages 265-277 *in* Sexual selection: testing the alternatives (J.W. Bradbury and M.B. Andersson Eds.). John Wiley, London.

Perrins, C.M. 1970. The timing of birds' breeding seasons. Ibis. 112: 242-255.

Petrie, M. & Møller, A.P. 1991. Laying eggs in others' nests: intraspecific brood parasitism in birds. Trends in Ecology and Evolution. 6: 315-320.

Pike, T.W. & Petrie, M. 2003. Potential mechanisms of avian sex manipulation. Biological Reviews of the Cambridge Philosophical Society. 78: 553-574.

Poulton, E.B. 1890. The Colours of Animals. 2nd ed. Kegan Paul, London.

Qvarnström, A. & Forsgren, E. 1998. Should females prefer dominant males? Trends in Ecology & Evolution. 12: 498-501.

Rohde, P.A., Johnsen, A., Lifjeld, J.A. 1999. Female plumage coloration in the bluethroat: no evidence for an indicator of maternal quality. Condor. 101: 96-104.

Roulin, A., Dijkstra, C., Riols, C., Ducrest, A.L. 2001. Female- and male-specific signals of quality in the barn owl. Journal of Evolutionary Biology. 14: 255-256.

Roulin, A., Ducrest, A.L., Balloux, F., Dijkstra, C., Riols, C. 2003. A female melanin ornament signals offspring fluctuating asymmetry in the barn owl. Proceedings of the Royal Society of London. 270: 167-171.

Rohwer, S. & Rohwer, F. C. 1978. Status signaling in harris sparrows: experimental deceptions achieved. Animal Behaviour. 26: 1012-1022.

Royle, N.J., Surai, P.F., Hartley, I.R. 2001. Maternally derived androgens and antioxidants in bird eggs: complementary but opposing effects? Behavioral Ecology. 12: 381-385.

Saino, N., Bertacche, V., Ferrari, R.P., Martinelli, R., Møller, A.P., Stradi, R. 2002. Carotenoid concentration in barn swallow eggs is influenced by laying order, maternal infection and paternal ornamentation. Proceedings of the Royal Society of London. 269: 1729-1733.

Saino, N., Martinelli, R., Biard, C., Gil, D., Spottiswoode, C.N., Rubolini, D., Surai, P.F., Møller, A.P. 2007. Maternal immune factors and the evolution of secondary sexual characters. Behavioral Ecology. 18: 513-520.

Sandell, M.I. 1998. Female aggression and the maintenance of monogamy: female behaviour predicts male mating status in European starlings. Proceedings of the Royal Society of London. 265: 1307-1311.

Sandell, M.I. & Smith, H.G. 1997. Female aggression in the European starling during the breeding season. Animal Behaviour. 53: 13-23.

Schluter, D., & Price, T. 1993. Honestly, perception and population divergence in sexually selected traits. Proceedings of the Royal Society of London. 253: 117-122.

Schwabl, H. 1993. Yolk is source of maternal testosterone for developing birds. Proceedings of the National Academy of Science of the United States of America. 90: 11446-11450.

Schwabl, H. 1996. Maternal testosterone in the avian egg enhances postnatal growth. Comparative Biochemistry and Physiology Part A: Physiology. 114: 271-276.

Schwabl, H. 1996. Environment modifies the testosterone levels of a female bird and its eggs. Journal of Experimental Zoology. 276: 157-163.

Senar, J.C. 2006. Color Displays as Intrasexual Signals of Aggression and Dominance. Pages 87-136 *in* Bird Coloration: Function and Evolution (G.E. Hill and K.J. McGraw Eds.). Harvard University Press, Cambridge, MA.

Slagsvold, T. 1993. Female-female aggression and monogamy in great tit (*Parus major*). Ornis Scandinavica. 24: 155-158.

Stephens, M.L. 1982. Mate take-over and possible infanticide by a female northern jacana (*Jacana spinosa*). Animal Behaviour. 30: 1253-1254.

Surai, P. F. & Speake, B. K. 1998. Distribution of carotenoids from the yolk to the tisues of the chick embryo. Journal of Nutritional Biochemistry. 9: 645-651.

Svensson, P.A., Pélabon, C., Blount, J.D., Surai, P.F., Amundsen, T. 2006. Does female nuptial coloration reflect egg carotenoids and clutch quality in the two-spotted goby (*Gobiusculus flavescens* Goblidae)? Functional Ecology. 20: 689-698.

Török, J., Hargitai, R., Hegyi, G., Matus, Z., Michl, G., Péczely, P., Rosivall, B., Tóth, G. 2007. Carotenoids in the egg yolks of collared flycatchers (*Ficedula albicollis*) in relation to parental quality, environmental factors and laying order. Behavioral Ecology and Sociobiology. 61: 541-550.

Thorpe, W.H. 1972. Duetting and Antiphonal Song in Birds: It's Extent and Significance. Sub-Department of Animal Behaviour, Cambridge, England.

Von Schantz, T., Bensch, S., Grahn, M., Hasselquist, D., Wittzell, Håkan. 1999. Good genes, oxidative stress and condition-dependent sexual signals. Proceedings of the Royal Society of London. 266: 1-12.

Wallace, A. R. 1878. Tropical Nature, and Other Essays. MacMillan and Co, London.

Weastneat, D.F. 2006. No evidence of current sexual selection on sexually dimorphic traits in a bird with high variance in mating success. American Naturalist. 167: E171–E189.

Weatherhead, P.J. & Robertson, R. 1979. Offspring quality and the polygyny threshold: "the sexy son hypothesis." The American Naturalist. 113: 201-208.

Weiss, S.L., Kennedy, E.A., Bernhard, J.A. 2009. Female specific ornamentation predicts offspring quality in the striped plateau lizard, *Scleroporus virgatus*. Behavioral Ecology. 20: 1063-1071.

Whittingham, L.A. & Schwabl, H. 2002. Maternal testosterone in tree swallow eggs varies with female aggression. Animal Behaviour. 63: 63-67.

Wingfield, J.C , Hegner, R.E., Dufty Jr., A.M., Ball, G.F. 1990. The "challenge hypothesis": theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. The American Naturalist. 136: 829-846.

Wittenberger, J.F. 1981. Animal Social Behavior. Duxbury, Boston.

Wolfenbarger, L.L. 1999. Red coloration of male northern cardinals correlates with mate quality and territory quality. Behavioral Ecology. 10: 80-90.

Yasukawa, K. & Searcy, W.A. 1982. Aggression in female red-winged blackbirds: a strategy to ensure male parental investment. Behavioral Ecology and Sociobiology. 11: 13-17.

Zahavi, A. 1975. Mate-selection- a selection for a handicap. Journal of Theoretical Biology. 53: 205-214.

Zuk, M. & Johnsen, T.S. 1998. Seasonal changes in the relationship between ornamentation and immune response in red jungle fowl. Proceedings of the Royal Society of London. 265: 1631-1635.